Abstracts of Presentations

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The number above an abstract corresponds to its designation in the program of the 1990 APS Annual Meeting in Grand Rapids, MI, August 4-8. If a presentation was not given at the meeting or was published in the *Canadian Journal of Plant Pathology*, the abstract is not printed among the following pages.

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A1

DETECTION AND COMPARISON OF ASTER YELLOWS STRAINS BY MONOCLONAL ANTIBODIES. K. D. Yang and T. A. Chen, Department of Plant Pathology, Rutgers University, New Brunswick, NJ 08903.

Monoclonal antibodies against the aster yellows mycoplasma-like organisms New Jersey strain (AY-NJ) and the aster yellows Eastern strain (AY-E) have been used to detect disease in AY infected plants. Five isolates partially purified from AY-NJ, AY-E, AY-Western, AY-Minnesota, and AY-Wisconsin infected lettuce have been compared with each other using AY-NJ monoclonal antibodies (Mabs), AY-E Mabs, and Maize Bushy Stunt Mabs. In enzyme-linked immunosorbent assay (ELISA) and immunofluorescent assay (FA), AY-NJ Mabs strongly react with AY-NJ, AY-E, AY-Minnesota, and AY-Wisconsin but not with AY-Western. AY-E Mabs react with all five isolates. However, Maize Bushy Stunt Mabs only react with AY-Western strain but not with other AY strains. This suggests that the AY-Western strain has different antigenic determinants from other AY strains and also suggests a relationship between Maize Bushy Stunt and AY-W. We will confirm this result by western blot analysis.

A2

MONOCLONAL ANTIBODIES AGAINST TOMATO BIG BUD MYCOPLASMALIKE ORGANISM (MLO) DISTINGUISH A GROUP OF INTERRELATED MLO STRAINS WITHIN THE "ASTER YELLOWS" STRAIN CLUSTER. I.-M. Lee, R. E. Davis, and H.-T. Hsu. Agricultural Research Service, USDA, Beltsville, MD 20705.

Monoclonal antibodies (McAbs) raised against tomato big bud MLO were employed in dot immunobinding assays. The McAbs reacted only with strains in the aster yellows (AY) MLO strain cluster, and not with any of several other MLOs (including elm yellows, ash yellows, western X, clover proliferation, and potato witches' broom MLOs). However, reactions with AY strain cluster MLOs varied with strain of MLO. None of the McAbs reacted with certain well-characterized strains of AY MLO, including strains termed MAY (Maryland aster yellows), DAY, MN, OK3, NYAY, SAY, and TLAY. However, all reacted with any of several other MLO strains previously termed "aster yellows", including strains OK1, NAY, NJAY, and AY27. These results are consistent with nucleic acid hybridizations in which two distinct groups were identified within the AY MLO strain cluster (1988. Mol. Plant Microbe Interact. 1:303-310).

A3

BEET LEAFHOPPER TRANSMITTED VIRESCENCE AND CLOVER PROLIFERATION MYCOPLASMALIKE ORGANISMS (MLOs): TWO NEW DISTINCT STRAIN TYPES. I.-M. Lee, R. E. Davis, and C. Hiruki[1]. Agricultural Research Service, USDA, Beltsville, MD 20705, USA, and [1]University of Alberta, Edmonton, Alberta, Canada T6G 2P5.

Molecular hybridization probes containing cloned DNA fragments from beet leafhopper transmitted virescence (VR) MLO or from clover proliferation (CP) MLO were prepared. The probes were employed in dot hybridizations against nucleic acid extracted from plants of Catharanthus roseus infected by VR or CP MLO or by one or another of ten different MLOs. Results from the hybridizations indicated that VR and CP MLOs are distinct from one another and are only distantly related to other MLOs, including aster yellows (AY), tomato big bud (BB), periwinkle little leaf (O-1), elm yellows (EY), and western X disease MLOs. The results are consistent with results from hybridizations with probes containing cloned DNA fragments from AY, BB, O-1, and EY MLOs, and with the concept that VR and CP MLOs are representatives of two new MLO strain clusters.

A4

ISOLATION AND MOLECULAR CLONING OF DNA FROM A MYCOPLASMA-LIKE ORGANISM (MLO) ASSOCIATED WITH WALNUT WITCHES' BROOM (WWB) DISEASE. J. Chen[1], C. J. Chang[1], R. Jarret[2], and N. Gawel[2]. [1]Department of Plant Pathology and [2]USDA-ARS, Plant Introduction Station, University of Georgia, Griffin, GA 30223.

Total DNA was extracted with CTAB buffer from freeze-dried petioles and leaves of walnut (Juglans nigra L.) showing severe symptoms of WWB disease. DNA of WWB MLO was separated from host DNA by centrifugation in CsCl. WWB MLO DNA and pUC18 were digested with Eco RI and Hind III, ligated and used to transform E. coli JM83. WWB MLO DNA clones were screened using [^{32}P]- labeled DNA from both healthy and diseased trees. MLO DNA inserts were verified by agarose gel electrophoresis and dot blots of healthy and diseased walnut DNA with [^{32}P]-labeled cloned plasmids. Using dot hybridization, at least one WWB MLO DNA clone (pWWB14 - 1.9 kb) hybridized strongly to DNA extracted from tissues of WWB infected trees, but only weakly to that from pecan bunch MLO, and not at all to blots of periwinkle infected with Western X, BLATV, eastern and severe western aster yellows MLOs, and Spiroplasma citri.

A5

DISTRIBUTION AND MULTIPLICATION OF WESTERN ASTER YELLOWS MLOs IN CATHARANTHUS ROSEUS. C. R. Kuske and B. C. Kirkpatrick. Department of Plant Pathology, University of California, Davis, CA 95616.

The distribution and multiplication of two AY-MLO strains in C. roseus plants were monitored over a ten week period. Plants were graft-inoculated with either the severe (SAY-MLO) or dwarf strain of AY-MLO. DNA was isolated from plant tissues and hybridized with ^{32}P-labelled DNA probes derived from chromosomal or plasmid DNA of SAY-MLO. Relative concentration of MLOs in different regions was quantified by scintillation counting of hybridized DNA. Colonization patterns of the two AY-MLO strains were similar. MLOs were first detected in grafted shoots about two weeks before symptoms appeared. The MLOs moved from grafted shoots into ungrafted shoots, and then systemically throughout the plant. Distribution and concentration of MLOs correlated directly with expression of virescence and proliferation symptoms. MLO concentrations were highest in symptomatic, actively growing shoots and lowest in roots.

A6

GIBBERELLINS AND THEIR POSSIBLE INVOLVEMENT IN THE HOST INDUCTION RESPONSE OF THE BEET LEAFHOPPER TRANSMITTED VIRESCENCE

AGENT. *D. A. Golino, N. Fang, *A. Yen, and L. Rappaport. *USDA-ARS, *Department of Plant Pathology and Department of Vegetable Crops, University of California, Davis, CA 95616.

Research has been undertaken to determine whether changes in gibberellin (GA) levels are involved in the host induction response (HIR), the precocious flowering brought about by infection with a mycoplasma-like organism, the beet leafhopper transmitted virescence agent (BLTVA). Quantitative and qualitative analysis of endogenous GAs in rosette healthy, bolting healthy and bolting BLTVA-infected Raphanus sativus indicated that GAs are elevated in the infected plants. Infection of GA deficient dwarf mutants (GA-1) of Arabidopsis thaliana with BLTVA did not restore the normal phenotype. This mutant is reported to be deficient in ent-kaurene synthase. Thus, although elevation of GA levels may be involved in the HIR, these experiments indicate that BLTVA can not compliment deficiencies in the synthesis of ent-kaurene, an important regulated step in GA synthesis.

A9

SENSITIVE DETECTION OF MYCOPLASMALIKE ORGANISMS (MLOs) BY POLYMERASE CHAIN REACTIONS (PCR). D. A. Schaff, I. -M. Lee and R. E. Davis. Microbiology and Plant Pathology Laboratory, Agricultural Research Service, USDA, Beltsville, Maryland 20705.

Molecular hybridization of specific cloned DNA probes has been used for a rapid means of detection of MLOs. To refine the sensitivity of detection of MLOs, polymerase chain reaction (PCR) was employed. Oligonucleotide primer sequences were derived from partial sequences of cloned aster yellows (AY) MLO DNA fragments and were synthesised for use as PCR primer sets. PCR reaction products were visualized in ethidium bromide-stained agarose gels. Forty cycles of PCR enabled the visualization of MLO-specific bands when as little as 2 pg total nucleic acid from infected plants was present in the reaction mixture. The use of PCR with various MLO-specific primer sets allowed differentiation of related MLOs.

A10

ISOLATION AND SIZE ESTIMATION OF WHOLE CHROMOSOMES FROM MYCOPLASMA-LIKE ORGANISMS. H. Neimark, SUNY Health Sciences Center, Brooklyn, NY 11203 and B. C. Kirkpatrick, University of California, Davis, CA 95616.

MLO and mitochondria-enriched fractions obtained from healthy and infected plants and insects were embedded in agarose blocks. The blocks were treated with SDS, proteinase K and X-ray irradiation to product MLO chromosomes containing random, single breaks. Whole MLO chromosomes were readily separated from contaminating host nucleic acids by pulsed field gel electrophoresis. The identity of MLO chromosomes was confirmed by Southern blot analysis. Compared to the mobilities of yeast markers and Mollicute chromosomes, the estimated MLO chromosome sizes of two X-disease strains and the beet leafhopper transmitted virescence agent ranged from 640 to 675 kbp while the western aster yellows was approximately 1185 kbp. Chromosome sizes of nonphytopathogenic MLOs present in several healthy leafhopper species ranged from 500 to 550 kbp.

A11

DETERMINATION OF WALL-DEGRADING ENZYME PRODUCTION BY Fusarium moniliforme AND F. graminearum USING ELISA AND IMMUNOGOLD LABELING. L. R. Todd, Department of Plant Pathology, University of Minnesota, St. Paul.

The production of wall-degrading enzymes of two corn-infecting Fusarium species were assayed. When two isolates each of F. moniliforme and F. graminearum were grown in cellulose nutrient broth (CNB) and in glucose ammonium-nitrate asparagine medium (GANAsn) for 4 and 6 days, ELISA testing of culture fluids of CNB-grown isolates indicated that endo-1,4-β-glucanase and 1,4-β-D-glucan cellobiohydrolase were present in all isolates. Lignin peroxidase activity was not detected in culture fluids from isolates grown in GANAsn; however, endo-1,4-β-xylanase was present in all isolates. Immunogold labeling of hyphae indicated that in all experiments the enzymes were associated with the cell wall and with the plasmalemma. In young, actively growing hyphae, gold particles were also observed within the cytoplasm. These results confirm that wall-degrading enzymes are produced by Fusarium species and suggest that these enzymes may be involved in infection of corn.

A12

BACTERIAL PROMOTER-LIKE SEQUENCE IN CLONED CHROMOSOMAL DNA OF MYCOPLASMA-LIKE ORGANISM (MLO) ASSOCIATED WITH CLOVER PROLIFERATION. S.J. Deng and C. Hiruki, Dept. of Plant Science, Univ. of Alta., Edmonton, Alta., Canada T6G 2P5.

Recombinant plasmids containing chromosomal DNA segments of MLO associated with clover proliferation (CP) were identified by dot- and Southern-blot hybridizations. The sequence of the cloned CP MLO DNA in pUC19 was determined by the dideoxy chain termination method using modified T_7 DNA polymerase and *Taq* DNA polymerase. A DNA segment of 191 base pairs consisted of only 17.8% G+C content. A bacterial promoter-like sequence was found in the sequenced DNA and an MLO promoter sequence was proposed. The RNA polymerase binding sites at -10 and -35 regions, the ribosome binding site, transcription and translation initiation sites were identified. The similarity of the -10 and -35 regions of the MLO promoter and the *Escherichia coli* promoter indicated that the two prokaryotes may have similar transcription regulation systems. The presumptive ribosome binding sequences in the MLO promoter may indicate that the 3'-OH terminus of the 16S rRNA of CP MLO is distinct from that of *E.coli*. The sequence between the two translation initiation codons may be involved in gene regulation.

A13

MOLECULAR CLONING AND ANALYSIS OF HMG-CoA REDUCTASE cDNAs FROM POTATO. B.A. Stermer, L.A. Edwards, N.L. Paiva, B.V. Edington and R.A. Dixon, The Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK 73402.

HMG-CoA reductase (HMGR) catalyzes the first reaction committed to isoprenoid biosynthesis and is involved in the coordinated regulation of sesquiterpenoid phytoalexin accumulation in potato. HMGR cDNA derived from the total RNA of arachidonic acid-elicited Kennebec tubers was amplified via the polymerase chain reaction and cloned into pSP72. Restriction digests and dideoxy sequencing defined 3 classes of cloned inserts 443, 296 and 155 base pairs long, each with a deduced amino acid sequence that is highly similar to Arabidopsis HMGR. Probing genomic Southern blots with the labeled cDNA inserts indicated the presence of a small gene family in potato. RNase protection experiments showed maximum HMGR expression in tuber disks at 18 through 36 h after elicitation. Work is in progress to characterize HMGR genomic clones.

A14

ISOLATION AND CHARACTERIZATION OF cDNA CLONES FOR PHENYLALANINE AMMONIA LYASE (PAL) FROM ALFALFA CELL SUSPENSION CULTURES. G. Gowri, K. Dalkin and R.A. Dixon, The Noble Foundation, Plant Biology Division, P.O. Box 2180, Ardmore, OK 73402.

L-phenylalanine ammonia-lyase (PAL) catalyzes the first step of the phenylpropanoid pathway in plants. PAL activity has been

shown to be induced dramatically in response to fungal elicitor in alfalfa. In order to investigate molecular mechanisms of this induction, we have recently isolated a near full-length cDNA clone (PAL-1) from an expression library using anti-alfalfa PAL antibody. Northern analysis using PAL-1 showed a maximum induction of the PAL mRNA in suspension cultures four hours after application of the fungal cell wall elicitor. The deduced amino acid sequence of this clone is highly similar to that of PAL clone(s) from bean. Southern hybridization of alfalfa genomic DNA with PAL-1 revealed the existence of a multigene family. Further, we have isolated several different PAL cDNA clones by rescreening the library with PAL-1, and characterization of these clones is in progress.

A15

DERIVATIVES OF CERCOSPORIN SHOW ALTERED TOXICITY. G. B. Leisman and M. E. Daub. Dept. of Plant Pathology, North Carolina State Univ., Raleigh, NC 27695-7616

We have synthesized four derivatives of cercosporin (CR) in order to understand structural requirements for toxicity. Dithionite-reduced cercosporin and its acetylated derivative were both unstable and could not be tested for toxicity. Noranhydro-cercosporin (NRC) and its methylated reduced derivative (TMNC) were tested for toxicity in the light to Neurospora crassa. Growth inhibition of N. crassa by CR, NRC and TMNC was 77, 98 and 74% respectively. There was no growth inhibition in the dark. A lipid peroxidation assay with methyl linolenate showed that NRC was twice as active as TMNC; CR was intermediate. Thus, NRC is more toxic than its reduced derivative and both require light for toxicity.

A16

CELL SURFACE REDOX POTENTIAL AS A POSSIBLE MECHANISM FOR CERCOSPORIN RESISTANCE. C. Cooperman Sollod, A. E. Jenns, and M. E. Daub. Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

The phototoxin cercosporin is toxic to plant cells and to many fungi, yet the producing fungus Cercospora is resistant. We hypothesize that resistance to cercosporin may result from a reducing environment at the cell surface which could reduce the toxin molecule or prevent generation of singlet oxygen. Toxicity of cercosporin has been shown to be directly related to light absorption. Dithionite reduction decreased light absorption by cercosporin approximately two-fold. Further, the addition of the reducing agents cysteine and sodium ascorbate to growth medium significantly decreased cercosporin toxicity to sensitive fungi. Nineteen tetrazolium dyes were tested as possible indicators of cell surface redox potential of five fungi differing in resistance to cercosporin. Dye reduction was media-dependent, and could not always be correlated with cercosporin resistance in different fungi.

A17

CHARACTERIZATION OF Rhizoctonia solani BY GAS-LIQUID CHROMATOGRAPHY OF CELLULAR FATTY ACIDS. J. Stevens Johnk and R. K. Jones, Dept. of Plant Pathology, University of Minnesota, St. Paul 55108.

Methyl esters of cellular fatty acids were analyzed by gas liquid chromatography using a fused silica capillary column (HP-5, 0.2 mm, 25 m). Preparation of the fatty acids from mycelia was accomplished by base saponification and organic solvent extraction. The major fatty acid found in six intraspecific groups (ISGs) of AG-1 and AG-2 was 18:2 cis 9,12 which constituted 68-80% of the whole-cell fatty acid content. This fatty acid along with 16:0 and 18:1 cis 9 account for greater than 93-97% of the C9-C20 fatty acids present. Smaller amounts of ten other fatty acids were consistently identified. Isolates within an ISG can be differentiated using principal component analysis of percent composition of the thirteen fatty acids identified.

A19

PREDISPOSITION OF PEANUT COTYLEDONS PRODUCED BY DROUGHT-STRESSED PLANTS TO ALTERED ISOZYME PATTERNS RECORDED DURING EARLY STAGES OF INFECTION BY *ASPERGILLUS* SPP. J.B. Szerszen and R.E. Pettit, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas 77843-2132.

Five peanut genotypes were grown under regimes of normal irrigation and drought stress. Drought stress lasted from 100 days after planting until harvest. Viable kernels from these plants (testa and embryonic axes removed) were inoculated with *Aspergillus flavus* or *A. parasiticus* (7×10^6 conidia per ml), incubated for 96 hr at dark, 95% RH, 32C, and sampled every 6 hr for electrophoretic analyses of isozymes using microprocessor-controlled discontinuous native-PAGE (gradient 8-25%) and IEF-PAGE (pI range 3-9). Drought stress did not change the total protein pattern of non-infected cotyledons; however, it predisposed the cotyledons to altered isozyme patterns exhibited during their colonization by the fungi, compared to infected cotyledons from plants grown under irrigation. These altered patterns include qualitative and quantitative changes of LAP, 6-PGD, G-6PD, MDH, ACPH and EST. Minor intergenotypic variations were recorded among genotypes tested.

A20

PATHOGENESIS-RELATED PROTEINS IN SOYBEAN INFECTED WITH COWPEA CHLOROTIC MOTTLE VIRUS. X.Q. Jiang* and C.W. Kuhn, Department of Plant Pathology, University of Georgia, Athens, GA 30602; *new address: Pioneer Hi-Bred International, Inc. Johnston, IA 50131.

Pathogenesis-related (PR) proteins were investigated in soybean infected with cowpea chlorotic mottle virus (CCMV)-strain S by electrophoresis, chromatography, ELISA and immunoblotting. We identified two types of soluble proteins in Bragg soybean which develops a hypersensitive reaction to CCMV infection. One type includes PR proteins S1, S2, and S3 which have common characteristics with previously reported PR proteins. They were associated with necrotic lesions, but not with induced resistance in uninoculated tissue. Another soluble protein (SP-H), not previously reported, was abundant in healthy plant tissue but decreased dramatically during lesion development. It has a relatively large molecular weight and a less acidic isoelectric point than PR proteins S1, S2, and S3.

A21

PROGRESS IN THE ISOLATION OF AN ACTIVE FACTOR FROM CUCUMBER PLANTS WHICH INDUCES SYSTEMIC RESISTANCE TO COLLETOTRICHUM LAGENARIUM. L. Fought and J. Kuć. University of Kentucky, Lexington, KY 40546

Many fungi, bacteria, and viruses form localized lesions and induce resistance throughout cucumber plants (systemic resistance). An extract was obtained from uninfected SMR-58 cucumber plants which induced resistance in SMR-58 to C. lagenarium. Leaves 1 and 2 were sprayed with the extract and activity was consistently associated with the appearance of chlorotic speckling on the sprayed leaves 1-2 days after spraying. Seven days after spraying, the upper unsprayed leaves were challenged with a conidial suspension of C. lagenarium. Plants sprayed with the extract had significantly less disease than unsprayed plants or plants sprayed with water. The compound(s) responsible for activity are soluble in water and boiling 95% ethanol and insoluble in ethyl acetate and diethyl ether. The compound(s) is stable to autoclaving and freeze-drying and has a molecular weight of ca. 180 D. Ion exchange chromatography indicates that the compound(s) is anionic.

A22

TRANSFORMATION OF A NON-PENETRATING MUTANT OF COLLETOTRICHUM LAGENARIUM TO WILD-TYPE PENTRATION. A.M. Bonnen, M. Murry, R. Hammerschmidt, and P. Hart. Botany and Plant Pathology Dept., Michigan State University, East Lansing, MI 48824-1312.

An ethylmethane sulfonate generated mutant(M-29) of Colletotrichum lagenarium was determined to be both attenuated in its ability to metabolize cellulose as a sole carbon source

and in its ability to penetrate intact cucumber tissue. A cosmid library was constructed using wild-type genomic DNA from C. lagenarium and the cosHygl vector. Transformation of the mutant with the cosmid library resulted in recovery of approximately 1000 hygromycin resistant colonies. The putative transformants were screened for the ability to utilize cellulose as the sole carbon source and for the ability to penetrate and cause disease in intact cucumber tissue. Several of the transformants were found to have regained the wild-type phenotype for these characteristics. All of the transformants contain vector DNA and appear to be identical as shown by Southern hybridization analysis. Attempts are being made to recover the transforming DNA by cosmid "rescue".

A24

INTERACTION BETWEEN PUCCINIA GRAMINIS F. SP. TRITICI AND PUCCINIA RECONDITA, GLOMUS INTRARADICES, AND GENOME IN WHEAT. C. B. Rempel and C. C. Bernier, Dept. of Plant Science, Univ. of Manitoba, Winnipeg, MB, Canada R3T 2N2.

The use of a model system using four wheat (Triticum aestivum) cultivars possessing differing numbers of resistance genes showed that the foliar pathogens Puccinia graminis f. sp. tritici (Pgt) and Puccinia recondita (Pr) reduced root colonization by the vesicular-arbuscular mycorrhizal (VAM) fungus Glomus intraradices. Simultaneous inoculation with a single virulent race of Pgt and of Pr significantly decreased VAM colonization in each of the four wheat cultivars as did simultaneous inoculation with an avirulent race of each pathogen. When virulent and avirulent races were inoculated onto the cultivars individually, colonization by VAM was reduced. Plant genome did not significantly affect VAM colonization of roots.

A25

EARLY INFECTION BY *HEMILEIA VASTATRIX* IN RESISTANT AND SUSCEPTIBLE SELECTIONS OF COFFEE AND IN A NON-HOST. T.A. Coutinho, F.H.J. Rijkenberg, and M.A.J. van Asch. Department of Microbiology and Plant Pathology, University of Natal, P.O. Box 375, Pietermaritzburg, 3200, South Africa

Fluorescence and scanning electron microscopy of early stages of infection by *H. vastatrix* in coffee and bean was conducted. On the leaf surface the sequence of events was similar for both host and non-host. A uredospore germinates from one to four germ pores but only one germ tube elongates and extends randomly over the epidermis. Exploratory branches form along its length. Once a stoma is encountered, a uniquely shaped appressorium forms either apically, or on a side branch, over one end of the stomatal slit. A distinct appressorial foot is wedged between the stomatal lips. In coffee, a torpedo-shaped substomatal vesicle (SSV) initial develops bilaterally from the apex of the infection wedge, while in bean, the infection wedge protrudes into the substomatal chamber and further development is solely unilateral. The SSV at 48 hours post-inoculation (hpi) is anchor-shaped. Haustorial mother cells are formed directly from the SSV on to subsidiary cells, or mesophyll cells. No differences, in appearance of these structures, were noted between resistant and susceptible coffee selections; a much branched mycelium ramifies through the intercellular spaces of the mesophyll cells 96hpi. In bean, the SSVs begin to collapse 48hpi.

A26

AN X. CITRI PATHOGENICITY GENE HAS DNA SEQUENCE SIMILARITY TO AVR GENES FROM X. MALVACEARUM AND X. VESICATORIA. S. SWARUP, R. DEFEYTER AND D.W. GABRIEL. Plant Pathology Dept., Univ. of Florida, Gainesville, FL 32611.

A 16 kb DNA fragmment (pSS10.35) obtained from a genomic library of X. citri was found to contain gene(s) important for the elicitation of the raised lesion phenotype of Asiatic citrus canker. Strains from pathovars alfalfae, citrumelo and cyamopsidis, compatible on grapefruit (cv. Duncan) leaves and containing the clone pSS10.35 induced hypertrophy of the host cells leading to the formation of raised canker-like lesions. Strains of X.c. pvs. malvacearum and phaseoli harboring pSS10.35 displayed significant avirulence activity and induced hypersensitivity when inoculated on their homologous host plants. Mutagenesis of pSS10.35 with Tn5-Gus showed that a 3.1 kb region was essential both for the increased pathogenicity on citrus and the avirulence activity on bean and cotton. Partial DNA sequence and restriction enzyme analyses revealed that the 3.1 kb region is highly similar to six avirulence genes from X.c. pv. malvacearum (avrB4, avrB101-102, avrb6, avrb7, avrBIn and $avrB_N$) and one from X.c. pv. vesicatoria (avrBs3). The possible role of some avirulence genes in pathogenicity will be discussed.

A27

X. MALVACEARUM AVR GENES ARE NOT ENTIRELY GENE-FOR-GENE SPECIFIC. R. DeFeyter and D.W. Gabriel. Plant Pathology Dept., U. of Florida, Gainesville, FL 32611.

Six X. campestris pv. malvacearum (Xcm) avr genes have been cloned and characterized by restriction enzyme digests, Tn5-GUS saturation mutagenesis and DNA sequence analyses. All six genes were found on a single 90 kb plasmid, all are larger than 3.2 kb in size, all are similarly organized and all are homologous to avrBs3 of X.c. pv. vesicatoria and to a pathogenicity gene from X. citri. Two of the genes appear to enhance symptom development on compatible hosts, and thus, like the X. citri gene, may play a role in virulence. The other four do not enhance symptom expression. Despite their similar structure, the six Xcm avr genes operate primarily in a gene-for-gene fashion. For instance, gene avrB4 gives a strong incompatible hypersensitive (HR) response in Xcm (but not E. coli) strains when inoculated onto cotton lines with the B4 resistance gene. Similarly, Xcm gene avrb6 gives a strong HR on cotton lines with the b6b6 gene. However, both avrB4 and avrb6 also elicit a weak HR on cotton lines with B1 or B2 (and without B4 or b6). Spontaneous mutations of the cloned genes have been obtained such that when the mutant clones are in Xcm, the strains elicit a strong HR on all cotton lines tested, including lines not known to carry resistance genes. The conservation of this class of avr gene in Xanthomonas implies a highly conserved phenotypic function, but their "recognition" of resistance genes appears to be a gratuitous phenotype, and not as specific as presented in the gene-for-gene hypothesis.

A28

AN AVIRULENCE FUNCTION FROM *PSEUDOMONAS SYRINGAE* PV. *TOMATO* IS LOCATED WITHIN A *HRP* CLUSTER. J. M. Lorang[1], C. A. Boucher[2], D. Dahlbeck[2], B. Staskawicz[2], and N. T. Keen[1]. [1]Dept. of Plant Pathology, University of California, Riverside 92521, and [2]University of California, Berkeley 94720.

Cosmid clone pPT10E9, previously cloned from *Pseudomonas syringae* pv. *tomato* PT23 (Kobayashi et al., PNAS 86:157, 1989), caused *P. syringae* pv. *glycinea* race 4 to elicit a hypersensitive response on all nine soybean cultivars tested. Restriction enzyme mapping and Southern blot analysis have revealed that the avirulence phenotype of clone pPT10E9 lies within or adjacent to a *hrp* cluster. The avirulence phenotype has been localized to an 11.35 kb DNA fragment located at one end of the *hrp* cluster. Characterization of this avirulence function and the possible role of the *hrp* clusters in determining host specificity will be discussed.

A29

CHARACTERIZATION OF POLYCLONAL ANTIBODIES TO THE HOST-SPECIFIC TOXIN VICTORIN. K. Akimitsu[1], L. P. Hart[1], and J. D. Walton[2]. [1]Department of Botany & Plant Pathology and [2]DOE-Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

Polyclonal antibodies against victorin, the host-specific toxin of Cochliobolus victoriae, were produced in rabbits immunized with victorin-BSA conjugates. The anti-victorin antibodies were purified from the serum by protein A column chromatography and characterized by indirect ELISA with goat anti-rabbit IgG alkaline phosphatase conjugate as a second antibody. The concentration of victorin inhibiting anti-victorin antibody binding by 50 % in the direct ELISA was 10 ng/ml. The lowest concentration of victorin detectable in the indirect ELISA was 10 pg/ml. About 60 ng of tritium-labelled victorin (5.3 mCi/mmol) was bound to 1 mg of anti-victorin antibodies in a binding assay. Using these antibodies, victorin binding to leaf tissue proteins of oats was detectable in western blots.

A30

DEVELOPMENT OF A HYPERSENSITIVE PHENOTYPE IN TRANSGENIC PLANTS EXPRESSING ELICITOR COAT PROTEINS OF TOBACCO MOSAIC VIRUS. J. N. Culver and W. O. Dawson, Department of Plant Pathology, University of California, Riverside, CA 92521

Recently, the induction of the N' hypersensitive resistance gene in *Nicotiana sylvestris* has been shown to be elicited by the coat protein of specific tobacco mosaic virus (TMV) mutants. In this study, both elicitor and non-elicitor coat protein open reading frames (ORF) were placed behind the CaMV 35S promoter and moved into the genome of *N. sylvestris*. Southern and Western blot techniques were used to show that transformed plants contained and expressed the coat protein ORFs. Plants

expressing a non-elicitor coat protein were slightly stunted but otherwise unchanged from the healthy, non-transformed phenotype of *N. sylvestris*. Plants expressing elicitor coat proteins showed mild to severe stunting and developed necrotic patches that eventually coalesced, collapsing entire leaves. This demonstrates that TMV elicitor coat proteins are singly responsible for the induction of the hypersensitive reaction in *N. sylvestris*.

A31

MONOCLONAL ANTIBODIES SPECIFIC TO PYTHIUM ULTIMUM AND PYTHIUM SPP. G. Y. Yuen and M. L. Craig. Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583

Monoclonal antibodies were produced for the identification and quantification of Pythium ultimum (PU). A crude preparation of PU cell wall material from Czapek Dox broth culture was used as the antigen. Initial selection of hybridomas was based on positive reactions in indirect-ELISA with a culture of PU and negative reactions with a strain of P. irregulare and of Phytophthora cinnamomi. Four antibodies were selected for further screening against 23 strains of PU, 35 strains of 16 other species of Pythium and 27 strains of other genera of soil fungi. One antibody (E5) was found to be specific to PU and to have the highest binding affinity. The remaining three exhibited some cross-reactivity to various species of Pythium. An antigen competition assay using E5 was developed that can detect PU specifically in soybean roots to 2.5 ug of PU protein per mg of root protein. Comparison of this protocol with root culturing methods to measure root infections by PU will be discussed.

A32

SERODIAGNOSIS OF FUNGI OF THE DIAPORTHE/PHOMOPSIS COMPLEX OF SOYBEANS, L. M. Brill and J. B. Sinclair, Dept. of Plant Pathology, University of Illinois at Urbana-Champaign, Urbana 61801-4709.

ELISAs were developed for detection and quantification of fungi of the Diaporthe/Phomopsis complex of soybeans. A culture filtrate of Phomopsis longicolla was used a immunogen to produce rabbit polyclonal antibodies. With cultured fungi, antibodies reacted strongly to isolates of the complex with limited cross reactivity to other soybean fungi. Cross reactivity was lower and sensitivity 100X higher in antigen capture ELISA than in antigen coat ELISA. The diagnostic capability of antigen capture ELISA was tested with soybean seeds. Extracts from pathogen-free seed were negative controls; extracts from seeds inoculated with members of the complex were positive controls. Detection of fungal antigens in seed tissues was successful, based on comparisons to agar plate bioassays. Monoclonal antibodies are being developed using BALB/c mice. The mice responded poorly to culture filtrate immunogens, but strongly to mycelial extracts of P. longicolla.

A33

PREDICTING YIELD LOSS FROM PHYTOPHTHORA MEGASPERMA F. SP. GLYCINEA IN SOYBEAN WITH A QUANTITATIVE IMMUNOASSAY OF PHYTOPHTHORA IN SOIL. A. F. Schmitthenner, Dept of Plant Pathology, The Ohio State Univ., Wooster, OH 44691.

Soil from 20 fields was assayed for Phytophthora antigen in April and August 1989 using an ELISA immunoassay developed by Agri-Diagnostics Assoc., Cinnaminson, NJ. Eight soil subsamples were pooled from 4 quadrats in each field and compared to a single bulked sample composed of 25% of each pooled sample. In addition, Phytophthora inoculum potential was bioassayed in each soil by evaluating seedling disease of soybean cv. Sloan. Yield of four alternate strips of metalaxyl treated and untreated soybeans was compared in each field to assess yield loss from Phytophthora. ELISA and bioassay Phytophthora values were higher in August soil than in April soil. Mean ELISA and bioassay values from the four quadrats collected in the April significantly predicted yield loss, while data from the August samplings did not, based on regression analysis. ELISA and bioassay values from the single bulked samples were poor predictors of yield loss.

A34

MONOCLONAL ANTIBODY-BASED IMMUNOASSAYS FOR DETECTION OF *PHYTOPHTHORA* SPP. IN PLANTS. J.H. Rittenburg, F.P. Petersen, S.A. Miller and G.D. Grothaus. Agri-Diagnostics Associates, 2611 Branch Pike, Cinnaminson, NJ 08077.

Sensitive laboratory and on-site enzyme-linked immunosorbent assays (ELISAs), based on a mixture of two monoclonal antibodies, have been developed to detect *Phytophthora* spp. in plant tissues. The two monoclonal antibodies provide a broad spectrum of reactivity within the genus. The laboratory assay, utilizing a 96-well microtiter plate format, has a lower limit of sensitivity of 25 ng protein/ml *P. megasperma* f. sp. *glycinea* mycelial extract. The laboratory assay can be completed in less than one hour. A rapid, on-site kit was developed utilizing a flow-through immunoassay format. Sensitivity of the on-site assay is similar to that of the laboratory assay. Testing requires no special equipment, and can be completed in 10 minutes. In a experimental system of azaleas, rhododendrons and junipers inoculated with *P. cinnamomi*, *P. cactorum* and/or *P. citrophthora*, both assays detected the pathogen in roots well before symptoms were visible above ground.

A35

USE OF PHYTOPHTHORA SPECIFIC IMMUNOASSAY KITS IN A PLANT DISEASE CLINIC. J. W. Pscheidt, J. Burket and S. Fischer. Dept. Botany & Plant Path, Oregon State University, Corvallis, OR 97331-2903.

Phytophthora specific ELISA immunoassay kits (Kit E, Agri-Diagnostics, Cinnaminson, NJ) were used in the diagnosis of plant specimens expressing root or crown rots, sent to OSU's Plant Disease Clinic. The following data were collected: field history, symptoms, fungi isolated on selective media, and color reaction of ELISA kits. Clinic samples with typical symptoms of Phytophthora root rot produced a positive reaction with the immunoassay as did pure cultures of *Phytophthora* sp. or some *Pythium* sp. isolated from these samples. An alyssum leaf sample with downy mildew (*Peronospora parasitica*) also produced a positive reaction. Other samples without typical *Phytophthora* symptoms and associated with a variety of other pathogens did not produce a positive reaction with the immunoassay. Cross reactivity with some *Pythium* sp. makes interpretation difficult, but when kit results are combined with field histories and symptomology, the immunoassays have proven to be a useful tool in the Plant Disease Clinic.

A36

DETECTION OF PHYTOPHTHORA PARASITICA IN INOCULATED AZALEA ROOTED CUTTINGS BEFORE AND AFTER METALAXYL TREATMENT USING ELISA, CULTURE, AND APPLE BAIT TECHNIQUES. J. M. Mullen, A. K. Hagan, N. K. Burelle, and B. J. Jacobsen. Auburn University, Auburn, AL 36849.

Healthy azalea rooted cuttings were inoculated with a zoospore suspension (400 zoospores/plant) of Phytophthora parasitica. ELISA (Agri-Diagnostics Associates), PARPH selective medium culture, and apple bait diagnostic techniques were performed on crown and root tissues immediately after inoculation and at 2-3 day intervals for 18-24 days. Soon after Phytophthora was detected, a metalaxyl drench (3.76 g. a.i./l) was applied. All 3 techniques detected P. parasitica soon after inoculation. ELISA consistently gave positive results throughout the study, but culture and bait techniques gave variables results after fungicide treatment. With all 3 techniques, non-inoculated control plants tested negative for P. parasitica, and inoculated, non-fungicide-treated plants gave consistently positive results except for the first few days after inoculation.

A37

AN IMPROVED MEDIUM FOR THE ASSAY OF SEPTORIA NODORUM FROM WHEAT SEED. Juju B. Manandhar and Barry M. Cunfer. Department of Plant Pathology, University of Georgia, Georgia Station, Griffin, GA 30223.

An agar medium was developed to improve the recovery of Septoria nodorum from wheat seed compared to the current best medium, oxgall agar. S. nodorum fluoresces under near UV light on oxgall agar, but it does not sporulate, and growth of other fungi is only partially suppressed. The new medium contains 10 g potato dextrose agar, 15 g agar, 1 g peptone, 1.5 g oxgall, 5 mg chloroneb, 5 mg dicloran, and 5 mg CuOH per L. Antibiotics (3.1 mg chloramphenicol, 3.1 mg erythromycin, and 12.5 mg tetracycline HCl per L) to control bacteria are added after autoclaving. This medium retains the fluorescence of S. nodorum and permits moderate sporulation within 7 days. Recovery of S. nodorum from seed is improved 15-35% and growth of other fungi is reduced >30% compared to oxgall agar.

A38

A DIAGNOSTIC IMMUNOASSAY TO DETECT CEREAL EYESPOT (FOOT ROT) DISEASE IN WHEAT. D. W. Saunders, D. M. Feindt, L.E.B. Johnson, C. M. Smith and J. W. Stave, Du Pont Co., P. O. Box 6101, Newark, DE 19714-6101.

A diagnostic immunoassay (ELISA) has been developed which detects presymptomatic infection of wheat by the fungal pathogen *Pseudocercosporella herpotrichoides* (Ph), the

causative agent of cereal eyespot disease. Rabbits were immunized with extracts of Ph cultures. Purified rabbit-anti-Ph was rendered monospecific by absorption with cross-reacting fungi. The monospecific antibodies form the basis of an antigen-capture ELISA. All strains of Ph tested in this assay react positively. No cross-reactions are observed with other common fungi, soil, or plant components. The ELISA detects 20 pg of a characterized Ph antigen preparation. An extract of symptomatic wheat stems diluted 10,000-fold reacts positively. In field trials, the ELISA detected the presence of Ph in inoculated fields prior to the appearance of symptoms. This detection occurred earlier and was more consistent than microbiological culture isolation methods.

A39

IMMUNO-DIAGNOSTIC ASSAY FOR CEREAL FOOT ROT: FIELD DEVELOPMENT RESULTS. C. M. Smith, L.E.B. Johnson, D. W. Saunders, D. A. Allison, D. M. Feindt, M. M. Joshi, and B. Labit, Agricultural Products Dept., Stine-Haskell Research Center, Du Pont Co., Newark, DE 19711.

A novel immuno-diagnostic assay rapidly and accurately identifies *Pseudocercosporella herpotrichoides* (foot rot or eyespot) presymptomatically in cereal plants. In extensive European field trials, the assay detected *P. herpotrichoides* in presymptomatic cereal stems as early as tillering. *P. herpotrichoides* antigen levels rose over the season, predicting increases in disease severity, and at harvest, correlated well with foot rot symptoms. Significantly higher *P. herpotrichoides* levels with limited symptoms were found in outer, senescent sheaths compared to inner stems. Assay results of stems from fields sprayed and unsprayed with foot rot fungicides confirmed visual assessment of disease control with fungicide treatment. These results emphasize the utility of this diagnostic to accurately identify foot rot, monitor disease progress, and assess disease control needs.

A40

FUNGAL DETERIORATION OF ELECTRONIC MEDIA. J. W. McCain and C.J. Mirocha, Dept. of Plant Pathology, Univ. of Minnesota, St. Paul, 55108.

Computer diskettes and magnetic audio, video, and computer tapes consist of a polyester plastic (Mylar) backing painted with a magnetic emulsion ("pigment") containing organic additives as lubricants (fatty acids), binders (polyurethanes), and dispersants (lecithin). To confirm that all of these media are susceptible to fungal deterioration, we inoculated sample media pieces by dipping them in spore suspensions from various common fungi. The Mylar and the polyvinyl chloride jackets and enamel hub rings from microdiskettes were only minimally colonized, but abundant hyphae grew on the surface emulsion of all the intact media. Eight brands of microdiskettes differed slightly in supporting fungal growth. Performance failure of consumer products likely results from fungi utilizing additives that accumulate on the media surfaces. This could cause aggregation of the metal oxides in the media or clogging of the recording heads of electronic hardware.

A41

ANASTOMOSIS GROUPING OF BINUCLEATE RHIZOCTONIA AGENTS USED FOR BIOCONTROL OF RHIZOCTONIA ROOT ROT OF SUGAR BEET. L. J. Herr, Dept. of Plant Pathology, Ohio State Univ., Wooster, OH 44691

Anastomosis groupings of 10 binucleate Rhizoctonia (BNR) agents used for biocontrol of Rhizoctonia crown and root rot of sugar beet in Ohio were investigated. The 10 BNR agents were paired with one another in all possible combinations. Positive anastomoses were observed in all combinations indicating the agents belong to a single anastomosis group. Selected BNR agents paired with CAG-1 through CAG-7 failed to anastomose indicating the agents do not belong in the CAG system. Two BNR agents were paired with 15 of Ogoshi's BNR anastomosis tester isolates. Both anastomosed with AG-B (others)=AG-B(o), but not with any other of the 15 AG testers, including AG-Ba and AG-Bb of the three intraspecific groups (ISG) within the AG-B group. All 10 BNR agents anastomosed with AG-B(o), indicating they all belong in this ISG, but none of the 10 anastomosed with either AG-Ba or AG-Bb. Variations in cultural characteristics were evident among the BNR agents belonging in AG-B(o).

A42

GENETIC ANALYSIS OF *GLOMERELLA GRAMINICOLA* [ANAMORPH *COLLETOTRICHUM GRAMINICOLA*]. Lisa J. Vaillancourt and Robert M. Hanau. Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, 47907.

A hermaphroditic, self-fertile isolate of *Glomerella graminicola* (Politis) [anamorph *Colletotrichum graminicola* (Ces.) Wils.] was isolated in 1989 from naturally infected corn in Indiana. It preferentially out-crossed with several self-sterile corn isolates of *C. graminicola* from North and South America. Crosses were performed on autoclaved corn leaves in a humidity chamber. A line of submerged perithecia was formed at the junction of the parent colonies after 2 to 3 weeks of incubation under continuous light at 18-20 C. Analysis of random ascospores from individual perithecia showed that markers segregated and recombined according to Mendelian laws. Self-fertile recombinants were obtained. Thus, strains carrying multiple markers may be created via transmission genetics. This study demonstrates that *C. graminicola* is amenable to classical genetic analysis.

A43

ULTRASTRUCTURAL KARYOTYPE FOR Puccinia graminis f. sp. tritici. E.W.A. Boehm[1], W.R. Bushnell[1], D.J. McLaughlin[2], A.P. Roelfs[1], and L.J. Szabo[1], 1) USDA/ARS Cereal Rust Laboratory, Dept. Plant Pathology and 2) Dept. of Plant Biology, University of Minnesota, St. Paul, MN 55108.

Accurate cytological determination of the karyotype (chromosome number and morphology) for many fungi is hindered by the cell wall, the small size of the nucleus and the presence, generally, of a large number of chromosomes. Past estimates of chromosome number in P. graminis f. sp. tritici have yielded n=6. We reevaluated the karyotype for this fungus from fusion nuclei in meiotic pachytene, using three-dimensional reconstructions from TEM serial sections of synaptonemal complexes. Pachytene occurred shortly after karyogamy in young, thin-walled, slightly melanized teliospores. Cells in pachytene were selected prior to TEM using epifluorescence microscopy of fixed, DAPI-stained teliospore protoplasts from which walls were mechanically removed. Three-dimensional reconstructions from TEM serial sections of synapsed homologues in teliospores from a single isolate (CRL 75-45-1781-3) indicated a chromosome number of eighteen.

A44

RELATEDNESS OF THREE DISTINCT POPULATIONS OF FUSARIUM GRAMINEARUM EXAMINED BY ALLOZYME AND RIBOSOMAL DNA POLYMORPHISMS. S. Brooks, T. D'Souza and G.C. Adams. Dept. of Botany & Plant Pathology, Michigan State University, E. Lansing, MI 48824

Allozyme polymorphisms and RFLPs of the nuclear ribosomal DNA were compared to investigate molecular evolution among three conspecific populations of F. graminearum. These populations are known to differ in pathogenicity, ecological niche, production of perithecia and mycotoxins, colony growth and morphology. Two populations, Groups 1 and 2, coexist in Australia and California and two, Types A (=Group 2) and B, coexist in the north central United States. Eleven enzymes showed strain specific allozyme polymorphisms among 13 isolates but no correspondence to Group or Type was evident. Similarly, divergence among the Groups and Types was not evident in analysis of rDNA cut with nine restriction enzymes. However, allozyme and mitochondrial DNA polymorphisms were useful in distinguishing parental factors inherited by hybrids formed by protoplast fusions between Type A and Type B stains.

A45

FERTILITY OF ISOLATES BELONGING TO *FUSARIUM* SECTION *LISEOLA* FROM CORN AND SORGHUM. **J. F. Leslie, C. J. R. Klittich, and C. Chaisrisook. Dept. of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan, Kansas 66506-5502.**

Within *Fusarium* section *Liseola* four mating populations were previously recognized (A-D). We have crossed more than 440 wild-collected isolates from the central and eastern United States with mating type testers representing these four populations. Isolates belonging to all four populations have been identified and account for approximately 50% of the isolates examined. Members of the A and D populations are recovered most commonly. Additionally, we have detected two more mating populations, E and F, that account for a further 20% of the population. Within the remaining 30% of the population there is preliminary evidence for three additional mating populations for which reliable testers are not yet available. Some isolates appear to be sterile regardless of conditions used for crosses. All fertile strains are competent as males, but many male fertile strains are female sterile. Isolates from the A and F populations are all morphologically *F. moniliforme*; isolates from the B and E populations are all morphologically *F. subglutinans*; and most isolates from the D population are morphologically *F. proliferatum*.

A46

SPECIFIC ANTIGENS FOR VEGETATIVE COMPATIBILITY GROUPS IN FUSARIUM OXYSPORUM F. SP. LYCOPERSICI. E.S.M. El-Kady, O.A.E. El-Dein, and R.W. Schneider, Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA, 70803-1720.

Immunoelectrophoretic techniques, including crossed immunoelectrophoresis (CIE), CIE with an intermediate gel, and CIE following antibody absorption in situ, were used to differentiate among 21 isolates of Fusarium oxysporum f. sp. lycopersici representing three vegetative compatibility groups (VCGs) and several single member VCGs (SMVCGs). Each of the isolates in multiple member VCGs had specific antigens that were detected within but not among VCGs, and three antigens were associated with pathogenicity and vegetative compatibility and were found in all multiple member VCGs. There were six, five, and five antigens that were specific for vegetative compatibility and unique to VCGs 0030, 0031, and 0032, respectively. No VCG-specific antigens were found for the SMVCGs. These results suggest that antigens responsible for heterokaryosis were lost in SMVCGs.

A47

CHILL-INDUCED FORMATION OF SCLEROTIA IN SCLEROTIUM ROLFSII.
C. D. Rawn, Seton Hall University, South Orange, NJ 07079.

On glucose-yeast extract agar (GYE) a Sclerotium rolfsii isolate grows from center to edge (40 mm) of a 90 X 15 mm dish in 3 days and then forms sclerotia (i) in a central cluster appressed to the agar and (ii) scattered in the aerial mycelium. In colonies chilled briefly (5 C, 4 hr) at 2 days (23 mm radius) and then returned to 24 C, the pattern is different. Fewer, or none, form in the center; a ring of sclerotia forms later just inside where the colony edge was at the time of the chill. A colony chilled twice before it reaches the dish wall forms the sclerotial ring just inside where the colony edge was at the time of the second chill, and few form in response to the first chill. Colonies chilled when the lead hyphae reach the dish wall produce many sclerotia at the wall, where many typically do not form. On a glucose-NH_4NO_3 agar (GN) most sclerotia form at the dish edge, unlike the pattern on GYE. Yet chilled colonies form sclerotial rings as on GYE, except that twice-chilled colonies on GN form the sclerotial ring before lead hyphae reach the wall. Chilling temporarily arrests growth and redirects sclerotial initiation.

A48

VARIATION OBSERVED IN RIBOSOMAL DNA OF PYTHIUM SPP. AGREES WITH ISOZYME ANALYSIS RESULTS.
W. Chen, J. W. Hoy, and R. W. Schneider. Dept. of Plant Path. and Crop Physiol., La. Agric. Exp. Sta., LSU Agric. Center, Baton Rouge, LA 70803.

Cluster analysis of isozyme data showed that some Pythium spp., such as P. arrhenomanes and P. graminicola or P. irregulare and P. spinosum, are similar. Several isolates of these species were selected for studies of ribosomal DNA. Polymerase chain reaction was employed to amplify regions of nuclear DNA that code for the 18s subunit and internal transcribed spacers (ITS). Variations were observed in ITS size. ITS for P. arrhenomanes and P. graminicola, P. irregulare and P. spinosum, and P. ultimum were about 850, 1000, and 920 bp, respectively. Each of the three ITS sizes showed a distinct banding pattern after restriction analysis. Present data show identical DNA sequences for a limited region of the 18s subunit, but restriction analysis revealed 18s differences between the first and last two ITS size groups. More 18s and ITS DNA sequences are being determined to compare these morphologically and isozymatically similar species.

A49

EFFECT OF POLARITY ON IN VITRO TUMOR FORMATION BY AGROBACTERIUM TUMEFACIENS AND NECROTIC RESPONSE OF GRAPE CULTIVARS.
M.I. Haque and R.N. Goodman, Department of Plant Pathology, University of Missouri, Columbia, MO 65211

Tumor formation by Agrobacterium tumefaciens (AT) was significantly increased when the grape stem explants were planted and inoculated basal end upwards in agar medium as compared with apex upwards. Catawba and Chancellor stem pieces inoculated basal end upwards exhibited a 40% increase in tumor occurrence and a 3-4 times increase in tumor size. The enhanced tumor production reflects basipetal auxin accumulation in the grape stem explants. This in vitro inoculation procedure provided an efficient method to study pathogenicity of At strains on a large scale in grape cultivars.

Some necrosis accompanied tumor formation following in vitro inoculation of grape stem explants. The necrotic response was more prominent where the shoots were inoculated in normal upward direction. In some cases, tumors were produced in early stages but they become necrotic later. It might be possible that higher auxin production in transformed cells lead to higher auxin oxidase activity and that might be responsible for this necrosis.

A50

CLASSIFICATION OF RHIZOMONAS SUBERIFACIENS, THE CAUSAL AGENT OF CORKY ROOT OF LETTUCE, IN SUPERFAMILY IV.
Kenneth N. Jochimsen and Ariena H.C. van Bruggen, Department of Plant Pathology, University of California, Davis, CA 95616.

The causal agent of corky root of lettuce was recently identified as Rhizomonas suberifaciens, a new gram-negative genus and species, with a characteristic fatty acid profile (including 2-OH-14:0 fatty acid), ubiquinone Q10, and a G+C content ranging from 58.2 to 59.5 mol%. The presence of ubiquinone Q10 indicated potential placement of the genus Rhizomonas in superfamily IV which contains the family Rhizobiaceae. This hypothesis was tested by determining the thermal melting profile of hybrids from ^{3}H-labeled ribosomal RNA of R. suberifaciens strain CA1 and chromosomal DNA of 15 species of gram-negative bacteria. The melting temperature for members of superfamily IV ranged from 65C to 80C whereas that for Pseudomonas fluorescens was about 60C. The data indicate that R. suberifaciens belongs to superfamily IV.

A52

DETECTION OF XANTHOMONAS CAMPESTRIS PV. PRUNI BY OUTER MEMBRANE PROTEIN PROFILES AND MONOCLONAL ANTIBODIES.
C.A. Goodman and M.J. Hattingh, Department of Plant Pathology, University of Stellenbosch, Stellenbosch 7600, South Africa.

Bacterial spot of stone fruit caused by Xanthomonas campestris pv. pruni (Xcp) sporadically causes heavy crop losses in certain fruit-growing areas of the south-western Cape Province of South Africa. Outer membrane proteins of five strains of Xcp, 15 reference strains of different pathovars of X. campestris, and common bacterial epiphytes isolated from stone fruit trees were separated by SDS-polyacrylamide gel electrophoresis. Two proteins from a strain of Xcp were injected singly into BALB/c mice. Monoclonal antibodies developed against Xcp were used to probe gels for homologous proteins.

A53

THE INFLUENCE OF TI PLASMID UPON ATTACHMENT OF AGROBACTERIA TO GRAPE CALLUS CELLS.
X. Pu and R. N. Goodman, Department of Plant Pathology, University of Missouri, Columbia, Missouri 65211.

Low-speed centrifugation, 500 rpm for 2 minutes, provided better recovery of bacteria than filtration through Miracloth and Whatman #1 filter paper. It allowed the separation of bacterial cells attached to grape callus cells from unattached ones. With this technique, various strains of Agrobacterium tumefaciens and A. radiobacter were tested for their attachment efficiency to grape tissue culture cells. Our data indicated that the presence of Ti plasmid in agrobacteria is critical for early attachment to grape cells. In addition, the strains carrying octopine type Ti plasmids are more aggressive than nopaline types, attaching to grape cells more rapidly and in greater numbers. On the other hand, bacteriocin-producing strain, A. radiobacter K84, which fails to suppress grape isolates, did not show any adhesion to grape callus cells during 5 hours incubation with grape cells. However, A. radiobacter HLB-2, which is capable of inhibiting grape isolates in vitro and in planta, exhibited a certain amount of adherence to grape cells. Its attachment performance tends to explain the biological control activity of A. radiobacter HLB-2 against grape isolates.

A54

SEROLOGICAL RELATIONSHIPS AMONG XANTHOMONAS CAMPESTRIS STRAINS ASSOCIATED WITH CITRUS BACTERIAL SPOT.
A. M. Alvarez, A. A. Benedict, and T. R. Gottwald. University of Hawaii, Honolulu, HI 96822; USDA, Orlando, FL 32803.

In contrast to Asiatic citrus canker-A strains that share a common epitope as revealed with a monoclonal antibody (mAb) A1, xanthomonads associated with citrus bacterial spot (CBS) in Florida were antigenically heterogeneous and shared epitopes with other X. campestris pathovars. Aggressive CBS strains reacted with a mAb designated CBS1, whereas moderately aggressive and nonaggressive strains reacted with mAb A1 and other mAbs generated to a moderately aggressive CBS strain, an X. c.

pathogen of ti, and X. c. dieffenbachiae. In Western blots, the lipopolysaccharide (LPS) pattern of all CBS strains were the same and were delineated from the LPS patterns of canker-A strains. When mAb data was combined with Restriction Fragment Length Polymorphism data and detached leaf bioassay data, CBS strains fell into two distinct groups consisting of aggressive strains in the first group and all others in the second. This conclusion is consistent with field epidemiological observations.

A55

GENETIC CHARACTERIZATION OF PATHOVARS OF XANTHOMONAS CAMPESTRIS CAUSING DISEASES ON CITRUS. D.S. Egel, J.H. Graham, and R.E. Stall. University of Florida, Citrus Research and Education Center, Lake Alfred, FL 33850.

Citrus bacterial spot, caused by Xanthomonas campestris citrumelo, is a new disease of citrus in Florida. The relationship between X. c. citrumelo and X. c. citri, causal agent of citrus canker, is uncertain. The S1 nuclease technique was used for DNA-DNA hybridizations between strains of the above pathovars and the type strain of X. c. campestris. Strain F1, an aggressive strain of X. c. citrumelo, was 56% similar to X. c. citri strain 9771, and 34% similar to X. c. campestris. Strain 9771 was 27% similar to X. c. campestris. In both DNA-DNA hybridization and pulsed field gel electrophoresis with rare-cutting restriction enzymes, strains of X. c. citrumelo were diverse. Although X. c. citri and X. c. citrumelo cause similar diseases on citrus, they are not closely related based on genetic analyses.

A56

OPTIMIZATION OF ELECTRICAL PARAMETERS FOR EFFICIENT ELECTROPORATION OF PLASMID DNA INTO *XANTHOMONAS CAMPESTRIS* PV. *ORYZAE*. T. J. White and C. F. Gonzalez, Department of Plant Pathology and Microbiology, Texas A&M University, College Station 77843.

Electroporation provides an effective transformation system for US strains of *Xanthomonas campestris* pv. *oryzae* (*Xco*). Initial attempts to introduce plasmid pUFR027 into *Xco* strain X37-2 with electroporation yielded moderate transformation efficiencies (10^4 transformants/ug DNA). Optimization of the two most critical parameters, electric field strength and pulse duration, indicated a large window of transformability for X37-2. A range of electric field strengths was investigated with pulse durations of 1, 5, and 10 msec. Using a 5 msec pulse, electric fields from 8.5 - 17.1 kV/cm resulted in efficiencies of 10^6 to 10^7 transformants/ug DNA, with percent survival ranging from 6 - 92%. Frequency of transformation for the applied electric fields averaged 2 X 10^{-2} transformants /survivor. When optimized conditions for X37-2 were tested on four different strains of *Xco*, lower efficiencies were observed and survivability was strain dependent. Results indicate optimization of electric field strength and pulse duration may be strain dependent for *Xco*. Effects of cell density and DNA concentration on transformation efficiencies for *Xco* were also investigated.

A57

EFFECT OF NUTRIENTS ON SURVIVAL OF ANTAGONISTIC PSEUDOMONAS SP. ON ALFALFA LEAVES. Y. Guevara and F. Lukezic, Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802.

Supplemental nutrients (peptone, yeast extract, dextrose, glycerol, King's B broth) were used in bacterial suspensions that were applied by an immersion technique onto alfalfa plans with different densities of leaf hairs. Plants were kept in a dew chamber for 48 h and then transferred to the greenhouse. Samples were taken at 0, 1, 2, 3, and 7 day intervals. Leaflets were washed with agitation 1/2 h in buffer and then plated on a selective medium in order to recover the antagonist. Bacterial survival was enhanced by King's B broth + dextrose, but no significant increase was observed with the other treatments. More bacteria were recovered from varieties with dense leaf hairs. Scanning Electron-microscopy studies showed that the bacteria were more numerous on the abaxial surface, in the crevices between cells, at the base of and directly on trichomes, and on main veins and stomatal surfaces.

A58

ARYL ß-GLUCOSIDASE ACTIVITY OF ENTEROBACTER CLOACAE ECCT-501. D.P. Roberts[1], C.J. Sheets[1], and J. Loper[2], [1]USDA, ARS, Beltsville, MD 20705, and [2]USDA, ARS, Corvallis, OR 97330.

The rhizosphere bacterium, Enterobacter cloacae strain EcCT-501, exhibits at least two distinct aryl ß-glucosidase activities in culture. The ß-glucosidase activities of EcCT-501 grown in M56 basal salts plus 0.5% glycerol were separated spatially into extracellular and cell-bound fractions and biochemically by carbohydrate inhibition assays. The ß-glucosidase activity associated with the cell-bound fraction was inhibited >2, >6, and >69 times as much by 25 mM glycerol, 100 mM mannose, and 2 mM glucose, respectively, as that associated with the culture supernatant. Both ß-glucosidase activities were strongly inhibited by 10 mM salicin. Two ß-glucosidase activities were detected in a genomic library of EcCT-501 constructed in the cosmid vector, pLAFR3. A cosmid clone, p6D2, and subcloned 0.9 kb and 4.3 kb SalI fragments from p6D2 each conferred ß-glucosidase activity to Escherichia coli DH5α. The potential role of glucoside catabolism in rhizosphere colonization will be evaluated in future experiments.

A59

GENETICS OF RESISTANCE TO PHYMATOTRICHUM OMNIVORUM IN UPLAND COTTON. K.M. EL-ZIK and P.M. Thaxton, Dept. of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843.

Seven cotton genotypes were crossed in a diallel mating scheme with no reciprocals. Parents, F_1 and F_2 progenies were field evaluated for resistance to Phymatotrichum omnivorum at Temple, TX for two yrs. Data were analyzed using Hayman's diallel and Griffing's combining ability procedures. Significant differences in number of plants killed were obtained among the parents, F_1's and F_2's four and five wks after appearance of first symptoms. Additive and non-additive (dominance and epistasis) effects were significant. Dominance was more important than additive effects in the variation for resistance to P. omnivorum. Only general and not specific combining ability was significant. Transgressive segregation was observed in the F_2 population for resistance to P. omnivorum. Broad sense heritability ranged from 0.31 to 0.46. Environment (year), growth stage, and maturity of the host had a significant effect on the contribution of the genetic components.

A60

DNA FINGERPRINTING OF *SEPTORIA TRITICI* ISOLATES REVEALS A HIGH LEVEL OF CLONAL DIVERSITY DISTRIBUTED ON A FINE SCALE IN A CALIFORNIA POPULATION. B. A. McDonald and J. P. Martinez. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas 77843-2132.

Genetic diversity in a California population of the wheat pathogen *Septoria tritici* was measured using variation at RFLP loci. A sample of 93 isolates collected from a single wheat field was assayed for restriction fragment length variation in nuclear DNA using 10 plasmid probes containing anonymous *S. tritici* DNA sequences. Nine of the probes hybridized to sequences present in only one or two copies in the genome and one probe (pSTL40) hybridized to highly variable sequences present in 4-13 copies in the genome. Isolates with identical multilocus haplotypes based on the nine single-copy probes usually had identical pSTL40 RFLP banding patterns, indicating that these isolates were clones and that pSTL40 is useful for DNA fingerprinting. pSTL40 identified 25 different clones among the 93 isolates, with identical clones clustered in the same location in the field. Possible mechanisms contributing to the high levels of RFLP variation associated with pSTL40 will be discussed.

A61

CHANGES IN THE GENETIC DIVERSITY OF *PHYTOPHTHORA INFESTANS* DURING AN EPIDEMIC IN CENTRAL MEXICO AS DETERMINED BY DNA FINGERPRINTS. J. M. Matuszak, S. B. Goodwin, W. E. Fry and *M. J. Villarreal-Gonzalez. Department of Plant Pathology, 334 Plant Science, Cornell University, Ithaca, NY 14853 and *National Potato Program, INIFAP, Toluca, Mexico.

Central Mexico is the center of diversity of *Phytophthora infestans* and is an area where it is known to reproduce sexually. We determined the identity of over 300 isolates of *Phytophthora infestans,* collected at regular intervals during an epidemic, from three sites near Toluca, Mexico. Two moderately repetitive DNA clones were used as fingerprints to determine the genetic diversity of the populations within fields, and how that diversity changed during an epidemic. At two of the sites isolates were collected from blocks of uniform cultivars, the third site consisted of a mixture of cultivars and isolates were collected from two cultivars. A large number of clones were identified at each site and sampling date.

A62

REASSESSMENT OF VEGETATIVE COMPATIBILITY GROUPS OF VERTICILLIUM DAHLIAE AND THE COMPATIBILITY OF ISOLATES FROM CALIFORNIA POTATOES. C. A. Strausbaugh, M. N. Schroth, A. R. Weinhold, and J. G. Hancock. Department of Plant Pathology, University of California, Berkeley, CA 94720.

Strains of Verticillium dahliae assigned to 16 vegetative compatibility groups (VCGs) by Puhalla and Hammel using micro-

sclerotial color mutants (Phytopathology 73:1305-1308) were reassessed using nitrate-nonutilizing mutants. The strains they assigned to VCG 1 were vegetatively compatible only with themselves. A second compatibility group was formed from strains they included in VCGs 2, 3, 5, 7, 8, 9, 13, and 14. The VCGs assigned to groups 4, 6, 12, 15, and 16 constituted a third group. The one strain assigned to VCG 10 was only self-compatible. The mutant obtained from VCG 11 was not compatible with strains from the other VCGs. Three isolates from potatoes growing in the Bakersfield region of California were compatible with strains from VCG 1. Twenty-seven isolates from potatoes growing in the Tulelake region, however, were vegetatively compatible only with strains from the group containing VCG 4.

A63

INHERITANCE AND A POSSIBLE MECHANISM OF RESISTANCE IN LETTUCE TO PLASMOPARA LACTUCAE-RADICIS. M.E. Stanghellini, R.W. Michelmore, S.L. Rasmussen, and G.J. Vandemark. First, third, and fourth authors, Dept. of Plant Pathology, University of Arizona, Tucson, Az. 85721. Second author, Dept. of Vegetable Crops. University of California, Davis, Ca. 95616.

The inheritance of resistance in lettuce to Plasmopara lactucae-radicis, a recently described root pathogen, was studied by segregation analysis. F2 progeny derived from a cross between a resistant (Cobham Green) and a susceptible (Calmar) cultivar segregated at approximately a 3 susceptible : 1 resistant ratio. These ratios suggest that the resistant phenotype is due to a homozygous recessive genotype at a single locus. Fungal colonization but no sporulation occurred on the roots of resistant plants. Microscopic observation of roots revealed a differential callose deposition between resistant and susceptible plants. Callose is deposited more abundantly around haustoria in roots of resistant plants. Callose deposition may be a mechanism of resistance in lettuce to P. lactucae-radicis.

A64

MITOCHONDRIAL INHERITANCE IN USTILAGO VIOLACEA. G. A. Wilch, and A. J. Castle. Dept. Biol. Sci., Brock University, St. Catharines, Ontario, Canada L2S 3A1

Restriction fragment length polymorphisms (RFLPs) among different geographic isolates of Ustilago violacea were used as markers for studying mitochondrial inheritance. Haploid cultures of opposite mating type from isolates that exhibit RFLPs were mated on artificial medium and induced to form dikaryotic hyphae by treatment with vitamin E. The hyphae grow until the vitamin E is exhausted and then revert to haploid uninucleate budding. The haploid progeny of these crosses were analysed for nuclear and mitochondrial genotype. All possible nuclear and mitochondrial combinations were recovered from most crosses. Most (70%) of the haploid progeny, however, had the mitochondrial genotype donated by the a2 parent. Possible explanations for this bias will be discussed.

A65

GENETIC CHARACTERIZATION OF RESISTANCE TO TOMATO MOSAIC VIRUS (ToMV) IN TOMATO SOMACLONES. S.S. Smith, Dept. of Biochemistry, University of Maine, Orono, ME 04469 and H.H. Murakishi, Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824-1312.

Six tomato somaclones, regenerated from a fully ToMV-susceptible line (GCRI-26), were selected for resistance to ToMV. The inheritance of this resistance appears to involve an incompletely dominant nuclear gene with a maternal effect, for each of the somaclonal lines. In crosses with isogenic tomato lines expressing known Tm resistance genes, it was determined that the somaclonal resistance was additive with Tm-1 but not with Tm-2. The type of resistance seen in the somaclonal lines was similar to that of the gene Tm-1 in that it suppressed symptom formation, limited virus multiplication, was not temperature sensitive and had similar virus strain responses. That the viral resistances generated through somaclonal variation resemble that of a viral resistance gene found in a wild tomato species (Tm-1) is notable.

A67

GENETIC DIVERSITY IN *FUSARIUM OXYSPORUM* F.SP. *LYCOPERSICI* USING RESTRICTION FRAGMENT LENGTH POLYMORPHISM. D. D. Pope, Department of Plant Pathology, University of Georgia, Athens, GA 30602

Random fragments of a *Pst*I library made from *Fusarium oxysporum* f.sp. *lycopersici* (FOL) were used to probe Southern blots of genomic DNA extracted from 15 FOL isolates that represented three known races, three vegetative compatibility groups, and nonpathogens. Restriction fragment length polymorphisms were detected using unique and repeat sequence probes. A binary system was adopted to code isolates with respect to the presence (1) or absence (0) of specific fragments. Genetic diversity values calculated from these binary codes indicated the degree of genetic similarity among isolates. Genetic diversity values were analyzed with UPGMA. Results indicated that the three races probably underwent stepwise adaptations to newly introduced resistance genes such that race 2 arose from race 1 and race 3 from race 2.

A68

DOUBLE-STRANDED RNA FROM *DIAPORTHE PHASEOLORUM* VAR. *CAULIVORA*, THE SOYBEAN STEM CANKER PATHOGEN. Y.H. Lee, J.P. Snow, G.T. Berggren, and R.A. Valverde. Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

Fifty-two isolates of *Diaporthe phaseolorum* var. *caulivora* (*Dpc*) originating from Louisiana (11), Mississippi (11), Florida (11), Georgia (11), Arkansas (2), Tennessee (1), Iowa (2), and Ohio (3) were collected and grown on potato dextrose broth for 2 - 3 weeks. Double-stranded RNA (dsRNA) was extracted by two cycles of cellulose (CF-11) chromatography and electrophoresed on 6% polyacrylamide gel. Among all isolates, 29 (56%) contained at least one molecule of dsRNA. Molecular weights of dsRNA from *Dpc* ranged from 0.23 to 3.0 x 10^6 daltons, and eight different band patterns were observed. Southern isolates exhibited all eight, whereas only one band pattern was observed in northern isolates. Cytoplasmic fractions from two isolates showed the same dsRNA band patterns as those from the total cell extracts. This suggested that dsRNA of *Dpc* might be located in the cytoplasm of the cell. The dsRNA of *Dpc* was not associated with virulence, toxin production, growth rate, or the activity of phenol oxidase. Isometric virus-like particles of about 30 nm in diameter were detected from one of the virulent isolates.

A69

EPIDEMIOLOGY OF SEEDBORNE XANTHOMONAS CAMPESTRIS PV TRANSLUCENS IN WINTER WHEAT. E.A. Milus and T.L. Kirkpatrick, Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701 and SWREC, Rt. 3, Box 258, Hope, AR 71801.

Bacterial stripe (black chaff) of wheat, caused by Xanthomonas campestris pv translucens (Xct), is an important disease in Arkansas. Research was conducted to evaluate seed treatments for reducing seedborne Xct and to determine the relationship between seedborne inoculum and epiphytic populations of Xct, disease severity, and yield loss. A rifampicin-resistant isolate of Xct was applied to seed of cultivar Florida 302. Infested seed was not treated or was treated with 1% acidified cupric acetate (ACA), Kocide SD (2.5 g/kg), or heat (70 C for 10 days), resulting in different levels of seedborne inoculum. The treated seed lots and a noninfested, nontreated check were planted at Foreman, AR, in October 1989. After 3 mo, plants from infested seed averaged 6 x 10^6 cfu/g of leaf tissue. Epiphytic populations of Xct averaged 10-, 100-, or 1000-fold lower on plants from infested seed treated with Kocide, ACA, or heat, respectively.

A70

RECEPTIVITY OF WINTER WHEAT LEAVES TO COLONIZATION BY A STRAIN OF PSEUDOMONAS FLUORESCENS. F. J. Gough and H. M. El-Nashaar, USDA-ARS, Plant Science and Water Conservation Laboratory, 1301 N. Western, Stillwater, OK 74075.

Fourteen winter wheat (Triticum aestivum) cultivars were compared for receptivity to leaf colonization by an antibiotic resistant strain (PFM2) of Pseudomonas fluorescens antagonistic to Septoria tritici. The cultivars, in replicated row plots, were inoculated with PFM2, suspended in water, in 1988 and 1989.

Areas under survival curves, calculated from estimates of PFM2 population densities on randomly selected flag and flag-1 leaves, were used as indicators of cultivar receptivity. Population densities were determined immediately after inoculation in both years, and thereafter at weekly intervals for a total of five times in 1988 and three times in 1989. Receptivity of cv. Vona to colonization by PFM2 was significantly (P=0.05) greater than that of any other cultivar in both years. According to Duncan's multiple range test, receptivity among the remaining 13 cultivars did not differ in 1988, but separated into three groups in 1989.

A71

EFFICACY STUDIES OF FIVE FUNGICIDES FOR CONTROLLING RHIZOCTONIA ROOT ROT OF WINTER WHEAT IN TEXAS. J. T. Mathieson, C. M. Rush, Texas Agricultural Experiment Station, P.O. Drawer 10, Bushland, TX 79012.

Rhizoctonia root rot has been found to be a problem associated with early planted wheat in the Texas Panhandle. Laboratory studies were conducted to determine the optimum conditions for disease development, and to test selected fungicides, Triadimenol, Imazalil, CGA169374, UBI1886, and Nusane + Nuzone, for efficacy. A known pathogenic isolate of Rhizoctonia solani, Ag-4, was tested at temperatures from 15-35 C to determine the optimum for disease. Plant emergence in infested soil decreased as the temperature increased, R^2=0.55. Tests were done both in vitro on amended PDA and in infested soil at 15, 25, and 35 C. Seed treated with Triadimenol had significantly greater emergence and dry matter production at each temperature compared to other treatments. Seed treated with Triadimenol had significantly less infection, both on the seed and coleoptile, for 25 days when compared to non-treated seed.

A72

TRIADIMENOL COMPARED TO CARBOXIN FOR CONTROL OF COMMON BUNT OF WHEAT. E. Williams, Jr. Plant Pathology Department, Oklahoma State University, Stillwater, OK 74078-9947.

Comparitive efficacy of triadimenol and carboxin against common bunt was evaluated at Perkins (north central) and Duke (southwest) OK using seed and soil inoculated with Tilletia laevis. Fungicides were applied on seed at 0.75, 1, 1.5 and 2 X labeled rates to determine minimum a.i. required to provide significant reductions in smutted heads. Smutted heads percentages were determined from smutted heads/healthy heads in 1.5 m rows with 4 replications in a randomized complete block. Smutted head readings were 65 and 61% for untreated checks at Perkins and Duke, respectively. Triadimenol at 0.15 g a.i./kg reduced bunt to 0.25% at Perkins, and provided total control at Duke. Carboxin at 1.3 g a.i./kg was the minimum rate required for total control at Duke, and at Perkins, 3.25% was the lowest level of bunt reduction. Both fungicides provided significant ($P \leq 0.01$) bunt reductions; however, triadimenol provided nearly complete control with less a.i. of chemical.

A73

COMPONENTS OF PARTIAL RESISTANCE TO SEPTORIA NODORUM AMONG BRAZILIAN SPRING WHEATS. A. M. PRESTES and B. M. CUNFER, CNPT/EMBRAPA, C. Postal 569. Passo Fundo-RS, Brazil and Department of Plant Pathology, University of Georgia, Griffin, GA 30223.

Thirty-two Brazilian spring wheats were evaluated for partial resistance to Septoria nodorum under greenhouse conditions at the Georgia Experiment Station. There were significant differences among the cultivars tested for all of the components of partial resistance studied. Among the 32 wheats tested the incubation period (IP) was shortest (5.8 days) in the susceptible cultivar BR 4 and longest (14.5 days) in the resistant cultivar CEP 14. The latent period (LP) was also shortest (13.7 days) on BR 4 and BR 12 and longest (19 days) on CEP 14. Disease progress was slowest on CEP 14, BR 32, PF 8722, PF 8723, PF 87584, CEP 17, CEP 19 and BR 8. All the cultivars above showed longer IP and LP except BR 8, which was among those with shortest IP and LP. Partial resistance to S. nodorum is available among Brazilian wheats and it may be traced to a few cultivars.

A74

COMPONENTS OF PARTIAL RESISTANCE TO SEPTORIA NODORUM IN EIGHT SOUTHEASTERN WINTER WHEAT CULTIVARS. Jennifer A. Yocum and Barry M. Cunfer, Department of Plant Pathology, University of Georgia, Griffin, Ga 30223.

Winter wheat cultivars were evaluated for resistance to Septoria nodorum under field conditions. Resistant cultivars Oasis and Saluda had longer incubation (IP) and latent periods (LP), and slower rates of lesion development than Holley and GA 100. Cultivars Andy and Gore were intermediate. IP and LP were progressively shorter from glumes to F-2 leaves. Greatest mean differences in IP and LP among cultivars were for glumes, 8.7 and 7.4 days respectively. Smallest mean differences in IP and LP among cultivars were for F-1 leaves, 5.7 and 1.8 days respectively. Area under the disease progress curve was significantly lower on resistant cultivars. There were significant correlations among components of partial resistance and associated components.

A75

COMPONENTS OF PARTIAL RESISTANCE TO SEPTORIA TRITICI IN MOROCCAN WHEAT. A. Farih and R. M. Hunger, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078.

Four spring wheats (Triticum aestivum L.) from the Moroccan Breeding Program (BT4, BT6, BT93, and BT323), that differed in level of resistance to Septoria tritici blotch, were quantitatively inoculated under growth chamber conditions to investigate the components of partial resistance to S. tritici Rob. ex. Desm. The components measured were incubation period, lesion number per gram leaf tissue, and percentage leaf area necrosis on seedlings. The wheat cultivars differed in responses to S. tritici with BT93 having the longest incubation period and lowest number of lesions, and BT4 the shortest incubation period and greatest lesion number. BT6 and BT323 were intermediate. Experiments are under way to further characterize these genotypes and to develop criteria to select breeding lines based on one or more components of resistance.

A76

COMPARISON OF THE SURVIVAL AND SAPROPHYTIC ABILITIES OF SEPTORIA TRITICI AND S. NODORUM. S.J. Wainshilbaum and P.E. Lipps, Dept. of Plant Pathology, The Ohio State Univer., Wooster, OH 44691.

The survival and saprophytic abilities of Septoria tritici and S. nodorum were tested on leaf tissue, filter paper and straw. The number of conidia/pycnidium and percent conidium germination were determined for each species in a field trial conducted from January to May, 1989. The number of conidia produced in pycnidia declined until early Feb. and Apr. for S. nodorum and S. tritici, respectively. Rates of decline in conidium production were not significantly different (P=0.05) between species. Filter paper and straw were inoculated and tested for change in weight at 2, 4 and 6 wk. Substrates inoculated with S. nodorum had a greater percentage of weight change (-15% on filter paper) than those with S. tritici (+.04% on filter paper), the latter was not significantly different from uninoculated controls. These results indicate that S. nodorum and S. tritici differ in their ability to survive on leaf tissue in the field and to grow saprophytically in culture.

A77

INHERITANCE OF RESISTANCE TO LEAF RUST AND RESPONSE TO CHLORIDE APPLICATION IN SEGREGATING WHEAT POPULATIONS. S.S.A. Rizvi, G.W. Buchenau and F.A. Cholick, Plant Science department, South Dakota State University, Brookings, SD 57007.

Precision controlled growth chamber experiments were conducted to study genetics of resistance to leaf rust in BC_1F_1, F_2BC and F_2 populations of cultivars SD2980, Shield, Prospect, Pak81, Pavon, Kohinoor83 and Sarhad 83. Linkage of genes with recombination values were estimated. In addition, response to low(10 ppm) and high (50 ppm) Chloride ions(CL) in F_2BC and F_2 plants of SD2980 and Shield studied. Tests of goodness of fit without CL indicated 1-3 genes for resistance to rust depending on cultivar and isolate. With CL, four genes were identified in SD 2980 with linkage in coupling in both cultivars. CL ions increased number of genes for resistance by one compared to plants without CL.

A79

PHYTOTOXINS FROM THE APPLE PATHOGEN BOTRYOSPHAERIA OBTUSA
P.V. Subbaiah, W.S. Chilton and T.B. Sutton, North Carolina State University, Raleigh, NC 27695.
Four isolates of Botryosphaeria obtusa(Schw.)Shoemaker,causal agent of black rot and frogeye leaf spot of apple were found to produce phytotoxins in culture, infected fruit and spore germination fluids(sgf). Mellein was the most abundant toxin isolated from the culture filtrate. Other toxins isolated were tyrosol, 4-hydroxymellein, 5-hydroxymellein and 4-hydroxybenzaldehyde. All toxins except 4-hydroxybenzaldehyde were present in B. obtusa-infected fruit, and mellein and 4-hydroxymellein could be detected in sgf. Seventeen apple cultivars were used in a leaf bioassay to determine phytotoxicity of different toxins. Of the apple cultivars, Supergold and Silverspur were highly sensitive to all toxins. Only three apple cultivars (Empire, Stayman and Firm Gold) showed resistance to mellein and 4-hydroxymellein. There was not a strong correlation between isolate pathogenicity and the amount of toxin production in culture.

A80

A RAPID BIOASSAY TO DETECT MONOGENIC RESISTANCE TO THE NORTHERN LEAF BLIGHT IN SWEET CORN PLANTS.
B. Bashan and Y. Levy. Department of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel

Phytotoxic substances were isolated from culture filtrates of *Exserohilum turcicum* grown in Fries medium, or from susceptible sweet corn plants infected with the fungus. The substances inhibit significantly chlorophyll a biosynthesis in susceptible corn seedlings, but not in corn seedlings containing Ht genes which confer resistance to *E. turcicum*. A linear relationship was found between the phytotoxic extract concentration and the inhibition of Chlorophyll a biosynthesis in susceptible plants. The response of ethiolanthic seedlings to the phytotoxic extract was tested on 35 sweet corn cultivars with or without Ht genes. The phytotoxic substances were extracted from culture filtrates of 5 different isolates of *E. turcicum*. A positive correlation was found between the aggressiveness of isolates and the phytotoxic activity.

A81

TEMPERATURE-DEPENDENT RESISTANCE TO DOWNY MILDEW IN MELON PLANTS: STRUCTURAL RESPONSES AND PROTEIN ANALYSIS.
M. Balass[1], Y. Cohen[1] and M. Bar-Joseph[2]. [1]Department of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel and [2]ARO, Volcani Center, Beth-Degan 50250, Israel.

Downy mildew caused by *Pseudoperonospora cubensis* is one of the most serious diseases of cucurbits in temperate regions. A susceptible reaction type was observed in resistant lines incubated at low temperatures (12 C), namely, enlarged lesions and abundant sporulation. The accumulation of callose, phenolic compounds and lignin-like materials in resistant lines incubated at 12 C were similar to that in susceptible plants incubated at 21 C. The molecular mechanism of the resistance at 21 C is not understood yet. SDS-PAGE analysis of *in vitro* translated proteins, soluble proteins, intercellular proteins and PR-proteins extracted from resistant and susceptible lines at 48 h after inoculation did not show any detectable differences. However, a 45 KD protein (P45) band was detected in the healthy resistant lines 31-10 (near isogenic) and PI124111F but not in the susceptible cultivar Hemed. In F1 hybrid (PI124111F x Hemed) the P45 was only partially expressed, but in later generations F3, F5 and F7 that are resistant, P45 was expressed as in the resistant lines. P45 was found in cotyledons and leaves but not in roots. Our intention is to use P45 as a genetic marker for resistance and to examine whether P45 is involved in the mechanism of resistance to *P. cubensis*. Supported by BARD

A82

LACK OF ASSOCIATION BETWEEN MANSONONE TOLERANCE AND VIRULENCE IN *OPHIOSTOMA ULMI*.
R.H. Proctor and E.B. Smalley. Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Mansonones are phytoalexins that accumulate in the xylem of elms infected with *Ophiostoma ulmi*, the incitant of Dutch elm disease. We examined the virulence of mansonone sensitive mutants of *O. ulmi* to determine if tolerance to mansonones is necessary for virulence on elm. Mutants were generated by treating yeast-like cells of *O. ulmi* with the mutagen nitrosoguanidine (NTG) and screening for increased sensitivity to 1,2-naphthoquinone, a mansonone analogue. Strains with increased sensitivity to 1,2-naphthoquinone were also highly sensitive to mansonones E and F. Both mycelial growth and germination of conidia of mutants were highly inhibited by the mansonones compared to the wild type. Three mutants were less virulent on American and Siberian elm compared to the wild type strain from which they were derived. However, two mutants were just as virulent as the wild type. Mutants were backcrossed to wild type strains to remove non-target mutations induced by NTG. After two backcrosses all mansonone sensitive strains were as virulent as the tolerant, wild type strains. These data suggest that the levels of mansonone tolerance exhibited by wild type strains of *O. ulmi* are not necessary for virulence on elm.

A83

INFECTION CUSHION DEVELOPMENT BY *RHIZOCTONIA SOLANI* KUHN ON SOYBEAN LEAVES AND FACTORS AFFECTING IT.
C.S. Kousik, J.P. Snow and G.T. Berggren Department of Plant Pathology and Crop Physiology, La. Ag. Expt. Sta., La State Univ. Ag. Center, Baton Rouge, LA 70803.

Infection cushion formation by *Rhizoctonia solani* (AG-1) on soybean leaves was studied with light and scanning electron microscopy. Infection cushion formation started 18 h after inoculation. An inverted "T" shaped foot formed laterally from the extending mycelium. The tips of the foot extended between the grooves formed by the epidermal cells and 28 h after inoculation additional inverted "T" shaped structures were produced which intermingled to form the infection cushion. Mucilagenous material binding these structures was also detected, and was greater with the web blight isolate (AG-1 IB) compared to the aerial blight isolate (AG-1 IA). Isolates of *R. solani* causing aerial and web blight did not form infection cushions on soybean leaf surface replicas of either resistant or susceptible cultivars. Infection cushions were formed by both aerial and web blight isolates on colloidon membranes over leaves of susceptible and resistant cultivars. The number of infection cushions was greater on susceptible cultivars than resistant cultivars. This evidence suggests that a chemical stimulus is needed for cushion formation. More infection cushions were formed by both aerial and web blight isolates on resistant or susceptible cultivars when inoculated plants were kept in continuous darkness compared to plants kept in continuous light. There was a highly significant correlation between number of infection cushions formed and disease severity.

A84

SPORE ATTACHMENT AND VIRULENCE OF NECTRIA HAEMATOCOCCA MATING POPULATION I (FUSARIUM SOLANI F. SP. CUCURBITAE).
M. J. Jones and L. Epstein. Department of Plant Pathology, University of California, Berkeley, CA 94720.

To study the role of spore attachment in the pathogenesis of the cucurbit rot pathogen Nectria haematococca, we isolated mutants with macroconidia that were deficient in attachment. After treatment with N-methyl-N'-nitro-N-nitrosoguanidine, we used an assay on polystyrene to enrich for attachment-deficient mutants. Two strains, called Att$^-$, produced macroconidia with a 50% reduction in attachment to polystyrene and to zucchini fruits. The Att$^-$ mutants and the wild type were indistinguishable in macroconidial morphology, percentage germination, growth rate, prototrophy and ability to mate. When macroconidia were inoculated into wounded zucchini fruits, the Att$^-$ mutants were as pathogenic as the wild-type strain. However, when macroconidia were inoculated onto the surface of unwounded zucchini, the mutants were less pathogenic. Thus, attachment of N. haematococca macroconidia to its host surface appears to be a virulence factor and spore attachment may play an important role in the epidemiology of the disease.

A85

GENETIC ANALYSIS OF VIRULENCE IN PHYTOPHTHORA MEGASPERMA F.SP. GLYCINEA.
R. G. Bhat[1], A. F. Schmitthenner[1], and B. A. McBlain[2], [1]Dept. of Plant Pathology, [2]Dept. of Agronomy, The Ohio State Univ., Wooster, OH 44691.

Inheritance of virulence against soybean in Phytophthora megasperma f.sp. glycinea (Pmg) was studied by repeated selfing of oospore progenies. Pmg races 1 and 4 were included in the study. Virulence of single-oospore isolates was tested with a hypocotyl inoculation method on a universally susceptible cultivar of soybean and three other differentials with single resistance genes Rps1, Rps1-c or Rps1-k. Pure breeding single-oospore isolates of races 4 and 1 were obtained after selfing for three and six generations, respectively. In progeny analysis of inbred isolates, race 1 segregated for race 1, avirulent and putative race 4. Race 4 segregated for race 4 and a few (<10%) other race(s). Most of the isolates of advanced generations took more time to kill soybean seedlings than the field isolates indicating possible expression of inbreeding depression.

A86

GENOMIC RELATIONSHIPS BETWEEN PEA ENATION MOSAIC VIRUS AND THE LUTEOVIRUSES.
S.A. Demler[1] and G.A. de Zoeten[2] [1]Department of Plant Pathology, University of Wisconsin, Madison, WI 53706 and [2]Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI, 48824.

Sequence comparisons of RNA 1 of pea enation mosaic virus (PEMV) and members of the luteovirus group have uncovered strong organizational and amino acid homologies between these groups. The putative polymerase of

PEMV is encoded by two overlapping out of frame reading frames, consistent with the frameshift hypothesis cited for luteovirus polymcrase expression. The C-terminal portion bears strong amino acid homology to both PLRV and BWYV, and contains all of the helicase and polymerase motifs characteristic of subgroup 2 of the luteoviruses. The coat protein is encoded on an internal open reading frame immediately followed by a second in frame sequence encoding the 29K aphid transmission subunit of PEMV. Both the arrangement and amino acid sequences of these proteins are homologous to the corresponding 3′ terminal open reading frames identified in the luteovirus group. This data further strengthens the taxonomic linkage between these two virus groups.

A87

A COMPENSATORY MUTATION OF THE SATELLITE RNA OF TOBACCO RINGSPOT VIRUS RESTORES SPONTANEOUS CIRCULARIZATION AND BIOLOGICAL ACTIVITY. Hans van Tol, Jamal M. Buzayan, and George Bruening, Department of Plant Pathology, University of California, Davis, CA 95616.

Tobacco ringspot virus supports the replication of a satellite RNA (sTobRV RNA). In vitro, the complementary strand, sTobRV(–)RNA, spontaneously ligates to form a circle. A four base pair stem in the sTobRV(–)RNA structure was modified by substituting the central two bases in one strand and, in another construction, in the other strand, such that in a third, doubly-mutated construction the two base pairs should be restored. Neither of the singly-mutated forms of the RNA increased in plants when co-inoculated with TobRV, and circularization of the corresponding sTobRV(–)RNAs was very limited. In the doubly-mutated sTobRV RNA, circularization and satellite RNA increase were restored. This result suggests that spontaneous circularization of sTobRV(–)RNA is necessary for the biological activity of the satellite RNA.

A88

GENETIC MAPPING OF SYMPTOM TIMING AND SEVERITY OF CUCUMBER MOSAIC VIRUS. Marilyn Roossinck and Peter Palukaitis. Department of Plant Pathology, Cornell University, Ithaca, New York, 14853.

Several strains of cucumber mosaic virus (CMV) show phenotypic differences in the time of appearance and severity of symptoms in zucchini squash (*Cucurbita pepo*). Using pseudo-recombinants between two prototype strains, Fny-CMV (a "fast" strain) and Sny-CMV (a "slow" strain), we have mapped the symptom differences to RNA 1. The "fast" strains also display a reduction in the levels of RNA 1, as compared to RNA 2; this alteration maps to both RNAs 1 and 2 of Fny-CMV, implying an interaction between the RNAs 1 and 2 (or their gene products). Making use of full length cDNA clones of Fny-CMV and partial cDNA clones of Sny-CMV, we have constructed a series of recombinant viruses in order to more precisely map the functional domains responsible for these phenotypic differences.

A89

THE 3'-TERMINAL HALF OF GENE VI OF CAULIFLOWER MOSAIC VIRUS INFLUENCES SYSTEMIC AND LOCAL RESPONSES IN SOLANACEOUS HOSTS. W. M. Wintermantel, E. P. Broglio,* and J. E. Schoelz, Dept. of Plant Pathology, University of Missouri, Columbia, MO 65211, and *Dept. of Plant Pathology, University of Kentucky, Lexington, KY 40546.

The 5' portion of gene VI of cauliflower mosaic virus (CaMV) is involved in determining the ability of the virus to systemically infect solanaceous hosts. Recombinants with three strains of CaMV have also demonstrated that sequences within the 3' portion of gene VI or the large intergenic region further affect host-viral interactions. Current studies using recombinant viruses have determined that the 3' portion of gene VI of CaMV strain D4 controls the appearance of necrotic local lesions on Nicotiana edwardsonii and contributes to the ability of selected CaMV recombinant viruses to move systemically in Nicotiana bigelovii. We have sequenced the 3' portion of gene VI of strain D4 and have compared this sequence to CM1841, a strain which fails to produce symptoms on N. bigelovii and N. edwardsonii. Within this region there are three amino acid differences between the two strains which may affect the appearance of necrotic lesions on N. edwardsonii and the development of systemic symptoms on N. bigelovii.

A90

A GENETIC ANALYSIS OF HOST RANGE DETERMINANTS OF CAULIFLOWER MOSAIC VIRUS STRAIN W260. S. G. Qiu and J. E. Schoelz, Dept. of Plant Pathology, University of Missouri, Columbia, MO 65211.

CaMV strains CM1841 and W260 cause systemic infection in a number of cruciferous hosts such as Brassica campestris. However, strain CM1841 does not infect Nicotiana bigelovii, a systemic solanaceous host for W260. To identify W260 sequences that determine systemic infection of N. bigelovii, we made exchanges between infectious CM1841 and W260 clones and the chimeric viruses were then tested on N. bigelovii. We found that genes I, IV, and VI must be derived from W260 for systemic infection of N. bigelovii. One DNA segment which contained the 3' half of gene V and the 19S promoter and a second DNA segment which contained genes II and III influenced the concentration of virus in systemically infected leaves. Further constructs are necessary to test the involvement of the 5' half of gene V and the large intergenic region.

A91

A POINT MUTATION IN THE COAT PROTEIN ABOLISHES APHID TRANSMISSIBILITY OF A POTYVIRUS. C. D. Atreya, B. Raccah, and T. P. Pirone. Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

A non-aphid transmissible (NAT) variant of tobacco vein mottling virus (TVMV) was used to test the hypothesis that the viral coat protein (CP) plays a role in determining aphid transmissibility. Comparison of the nucleotide sequences of an aphid transmissible isolate (TVMV-AT) with that of TVMV-NAT revealed a single nucleotide difference (G- ->A) at position 8445; this alters a single amino acid residue (G- ->E) at position 2747. A cDNA fragment representing the CP region of TVMV-NAT was substituted into the CP region of a full-length cDNA clone of TVMV-AT, and transcribed RNA was inoculated to tobacco plants. Aphids were unable to transmit the resultant hybrid virus which had the TVMV-NAT coat protein, although the concentration and infectivity of the hybrid virus in the source plants was as high as that of TVMV-AT. This is the first direct demonstration that a CP mutation affects aphid transmissibility of a potyvirus.

A92

DETECTION OF dsRNA SPECIES AND VIRUS-LIKE PARTICLES IN *UNCINULA NECATOR*. Ossmat I. Azzam, Dennis Gonsalves, Shigetou Namba, David M. Gadoury, and Roger C. Pearson. Cornell University, New York State Agricultural Experiment Station, Geneva, 14456.

In a study of the seasonal distribution of ds RNAs in grapevines infected with Rupestris Stem Pitting (RSP), we observed several unique dsRNA species by polyacrylamide gel electrophoresis that were associated with late-season foliar infection by *Uncinula necator*, but not with RSP. Homogenates of whole cleistothecia and conidia of *U. necator* yielded these same dsRNA species. Identity of dsRNA was confirmed by DNase and RNase treatments. Molecular weights of the dsRNA species ranged from 9.5×10^5 to 6.3×10^6 daltons. Spherical virus-like particles were found in negatively stained cell homogenates and thin sections of cleistothecia viewed under transmission electron microscopy. Further work will focus upon the distribution of the virus-like particles in fungal tissues and propagules, the frequency of their occurrence in U.S. isolates of *U. necator*, and their possible effects upon virulence, pathogenicity, and survival of the fungus.

A93

SIGNIFICANCE OF PHENAZINE BIOSYNTHESIS IN THE SURVIVAL OF FLUORESCENT PSEUDOMONADS IN SOIL HABITATS. M. Mazzola[1], R.J. Cook[2], L.S. Thomashow[2] and D.M. Weller[2], [1]Dept. Plant Pathology and [2]USDA-ARS, Washington State University, Pullman, WA 99161.

Pseudomonas fluorescens 2-79 and *P. aureofaciens* 30-84 suppress *Gaeumannomyces graminis* var. *tritici* (*GGT*), the causal organism of take-all of wheat. Phenazine antibiotics produced by these bacteria protect wheat roots against infection by *GGT*. Although many soil microorganisms produce antibiotics, the function of such compounds in soil habitats is largely unknown. The objective of this study was to determine the role of phenazine biosynthesis in the survival of fluorescent pseudomonads in soil and in the rhizosphere of wheat. Strains 2-79, 30-84, Tn5 mutants of these strains defective in phenazine production (Phz^-), and the mutant strains genetically restored for phenazine production (Phz^+) were introduced individually into Thatuna silt loam. Soil was planted to five successive cycles of wheat; soil and rhizosphere populations of the introduced bacteria were determined 20 days after each planting. Phz^- strains maintained significantly lower populations in the wheat rhizosphere and in soil than did their corresponding parent strains and their respective mutant strains restored to Phz^+. The results suggest that phenazine production contributes to the survival of 2-79 and 30-84 in soil habitats.

A94

DETECTION OF HERBICOLIN A IN CROWN AND ROOT TISSUES OF WHEAT SEEDLINGS AFTER INOCULATION WITH ERWINIA HERBICOLA. H.-J. Kempf, M. N. Schroth and G. Wolf*. Department of Plant Pathology, University of California, Berkeley, CA 94720.

The antibiotic herbicolin was detected in root and crown tissues of wheat seedlings after seeds had been inoculated with E. herbicola. Seeds with approximately 10^6 cfu/seed were sown into natural field soil and incubated at 21 C for 4 days. After emergence, the underground plant parts were washed clean from soil and bacteria, homogenized, and extracted with methanol. The concentrated extracts were spotted onto a TLC plate and herbicolin was detected on the plate by a bioassay with Candida albicans as a test organism. The highest concentration of

herbicolin was detected in the crown. The upper part of the root system contained a detectable amount of the antibiotic whereas the concentration herbicolin in the lower root parts was below the detection limit. The amount of extracted antibiotic was correlated with the number of cells of E. herbicola which decreased from the crown to the root tips.

A95

THE RELATIONSHIP BETWEEN MICROBIAL ACTIVITY AND SUPPRESSIVENESS OF CANADIAN SPHAGNUM PEAT TO PYTHIUM ROOT ROT OF POINSETTIA. M. J. Boehm and H. A. J. Hoitink, Dept. of Plant Pathology, The Ohio State University and Ohio Agricultural Res. and Dev. Center, Wooster, OH 44691.

Light (slightly decomposed) and dark (decomposed) Canadian sphagnum peat varied in suppressiveness to Pythium root rot of poinsettia caused by Pythium ultimum Trow. Root rot and population development of P. ultimum were highest in the dark sphagnum peat, intermediate in a lesser decomposed peat and suppressed in a very light, slightly decomposed sphagnum peat. Microbial activity, based on the rate of hydrolysis of fluorescein diacetate, was highest in the suppressive and lowest in the conducive peat potting mixes. When high levels of microbial activity persisted in the potting mix throughout the initial thirty five days after planting, root rot and Pythium population development were effectively suppressed for the remainder of the three month crop cycle. Using simple linear regression there was a significant interaction between suppressiveness of the various peat mixes and the corresponding microbial activity. Suppression was negated by heating the potting mixes at 60 C for 30 min and restored by adding small quantities of nonheated suppressive peat. This suggests that the effect was biological in origin.

A96

THE INHIBITION OF MACROPHOMINA PHASEOLINA BY FUSARIUM SOLANI. B. S. Corwin, Y. H. Sunboul, and T. D. Wyllie. Dept. of Plant Pathology, Univ. of Missouri, Columbia, MO 65211.

During root isolations from field grown soybeans with symptoms of sudden death syndrome (SDS), we observed inhibition zones between the blue-purple form of Fusarium solani and Macrophomina phaseolina on potato dextrose agar (PDA). We also noted that the isolation frequency of M. phaseolina from lateral and taproots was significantly lower from plants collected from an SDS-symptomatic field than from those collected from a nonsymptomatic field. Controlled experiments in vitro confirmed that F. solani inhibited the growth of M. phaseolina on PDA. F. solani also interfered with the germination of microsclerotia. We observed a reduction in the percent germination over time as well as a reduction in average germ tube length. Soybean plants were grown in autoclaved field soil reinoculated with known concentrations of M. phaseolina alone or M. phaseolina plus F. solani. Using CMR, a semi-selective medium for M. phaseolina, the isolation frequency of M. phaseolina from roots growing in the M. phaseolina plus F. solani soil was one half that of the isolation frequency from roots exposed to M. phaseolina alone. We conclude that F. solani inhibits M. phaseolina, although the mechanisms have not been elucidated.

A97

PATHOGENICITY OF AG-2-2 CULTURES OF RHIZOCTONIA SOLANI ISOLATED FROM BEANS AND SUGAR BEET ON BEAN SEEDLINGS. Cheryl A. Engelkes and Carol E. Windels, Department of Plant Pathology, University of Minnesota, St. Paul, 55108 and Northwest Experiment Station, Crookston, 56716.

Cultures of AG-2-2 of Rhizoctonia solani were isolated from rotted roots of beans and sugar beet. Nine AG-2-2 cultures were evaluated in the field for pathogenicity on two cultivars each of fababean, navy bean, pinto bean, and soybean (crops rotated with beet). For each treatment, 30 unifoliolate plants were inoculated with one AG-2-2 colonized corn kernel. Basal stems were rated using a 1 to 5 stem rot index (1=0%, 3=25-49%, 5=75-100% stem girdled). Fababeans had the highest rot index of 4.5, followed by soybeans 4.1, pinto beans 4.0, and navy beans 3.5. The two AG-2-2 cultures each from pinto bean and soybean gave an overall rot index of 4.8; two cultures from fababean 4.1; and three cultures from sugar beet 3.9. Based on these results, rotation of sugar beet with bean crops is not recommended.

A98

MANGANESE SEED CONTENT - AN AMELIORATING FACTOR FOR TAKE-ALL OF CEREALS. T.S. Roseman and D.M. Huber. Purdue University, W. Lafayette, IN 47907.

Manganese is a critical regulator of physiological defense reactions of plants to disease; and the severity of take-all root, crown, and foot rot of cereals has been correlated with such cultural practices as form of N, pH adjustment, and crop rotation which influence the availability of Mn. This study determined if the content of Mn in seed influences the severity of take-all. Five varieties of soft red winter wheat (*Triticum aestivum* L.) were grown under two widely different ecological conditions to modify their Mn seed content. Four varieties (Cardinal, Lincoln, Steele, Twain) differed by 20 to 30 ug g^{-1} in Mn seed content while the Mn seed content of one variety (Caldwell) was similar at both locations. All varieties were grown at three field locations in Indiana with natural infestations of *Gaeumannomyces graminis* var. *tritici*. Under these moderately-severe to severe disease conditions, plants with the higher Mn seed content were generally more vigorous, had an average of 11% less severe take-all (white heads), and yielded an average of 165 kg/ha more grain. No significant differences in vigor, yield, or take-all severity were observed with the variety grown from seed produced under widely different environments but with similar Mn seed content.

A99

INFLUENCE OF WINTER COVER CROPS ON SOIL POPULATIONS AND ISOLATION FREQUENCIES OF COTTON SEEDLING PATHOGENS. C. S. Rothrock and T. L. Kirkpatrick, Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701 and SWREC, Rt. 3, Box 258, Hope, AR 71801.

Winter legume cover crops reduce soil erosion and provide an environmentally attractive nitrogen source. The influence of legume cover crops in crop sequences with cotton on soilborne plant pathogen populations and colonization of cotton seedlings was examined at two locations. Colonization of seedlings by Rhizoctonia solani was increased following hairy vetch near Hope. However, no differences in soil populations of R. solani were detected. Soil populations of Thielaviopsis basicola and seedling colonization were reduced following hairy vetch cover crop treatments compared to winter fallow near Clarkedale. Soil populations of pathogenic Pythium spp. were greater at both locations following legume cover crops than fallow; however, no differences in isolation frequency were found. The influence of hairy vetch on the seedling disease complex depended on the prevalent pathogens at each location.

A100

DISEASE INCIDENCE-SEVERITY RELATIONSHIPS IN GRAPE POWDERY MILDEW. Robert C. Seem, David M. Gadoury, and Roger C. Pearson. Cornell University, NYSAES, Geneva, NY 14456.

Incidence (DI) and severity (DS) of grape powdery mildew (*Uncinula necator*) on the cultivar Rosette were examined in a 3-yr study. Treatments were: 3 early-, mid-, or late-season sprays of benomyl or triadimefon; and 2, 4, or 6 sprays during the season. DI was measured as infected leaves/shoot (%), and DS as infected leaf area/shoot (%). Early disease suppression resulted in less fruit infection than mid- or late-season suppression, possibly due to protection from ascosporic inoculum released before bloom and expression of resistance in maturing fruit. DI-DS relationships were affected by fungicide treatments, but were similar within treatments in all 3 years. Late-season sprays permitted DS to exceed 80% and resulted in curvilinear DI-DS relationships. Early- and mid-season sprays, and the 2- and 4-spray treatments reduced DS, and yielded a linear relationship. The 6-spray treatment kept DS at a low and constant level. However, in all treatments, DI in most plots eventually approached 100%. Neither triadimefon nor benomyl eliminated infection. Instead, DS was often reduced to the extent that DI-DS relationships were altered. We suggest that auto-infection (*sensu* Robinson) is fully operational in *U. necator* on grape.

A101

COTTONBALL DISEASE PROGRESS IN A WISCONSIN CRANBERRY FIELD. P. G. Sanderson and S. N. Jeffers. Department of Plant Pathology, University of Wisconsin, Madison 53706.

Spores of *Monilinia oxycocci* were collected and disease progress was monitored for two years in a commercial cranberry field with a history of cottonball. Shoots with symptoms were first observed on 7 June 1988 and 10 June 1989. Numbers of symptomatic shoots continued to increase for 17 and 27 days, at which time 18% and 12% of the shoots were blighted, in 1988 and 1989, respectively. Conidia were observed on shoots 7 and 6 days after initial symptoms were seen and were eventually produced on 76% and 85% of all blighted shoots in 1988 and 1989, respectively. Airborne conidia were trapped beginning 16 June, 10 days after initial bloom, for 26 days in 1988 and beginning 19 June, the first day of bloom, for 33 days in 1989. Conidium peaks began 32 and 31 days following the beginning of the ascospore peaks in 1988 and 1989, respectively. Although the onset of conidium dispersal differed relative to the time of first bloom, the bulk of spores caught coincided with full bloom in both years. In 1989, trap plants were placed in the field when conidia were abundant. No symptoms were observed on expanding shoots; however, an average of 36% of fruit on plants that were in bloom at the time became diseased. The incidence of diseased fruit did not increase after bloom in either year. Mycelium of *M. oxycocci* was detected in approximately 41% of the berries in 1988 and 35% of the berries in 1989.

A102

Incidence of eastern filbert blight in Oregon hazelnut orchards. J.N. Pinkerton[1], J. Griesbach[2], K.M. Theiling[3], and K.B. Johnson[3]. [1]USDA/ARS Hort. Crops Res. Lab., Corvallis, OR 97330, [2]Oregon Dept. Agric., Salem, 97310, and [3]Dept. of Botany & Plant Pathology, Oregon St. Univ., Corvallis, 97331-2902.

In 1986, hazelnut orchards with eastern filbert blight (EFB) were found near the northeast edge of the Willamette Valley, the major production area. From 1986 to 1990, 5,400 ha in 8 counties were surveyed. EFB was detected in the 3 most northern counties within 40 km south and west of the original detection area. EFB was found in 666 ha of commercial orchards, 43 ha of unmanaged orchards, and at 216 residential sites. Fifty one hectares of diseased orchards and symptomatic branches within lightly infected orchards have been destroyed. EFB was most severe in orchards planted with Ennis, Royal, and Davianna, whereas the major cultivar, Barcelona, appears moderately resistant. Secondary foci have developed around susceptible plantings. Within-orchard spread of EFB is mostly to the northeast, the direction of the prevailing winds.

A103

INFLUENCE OF TEMPERATURE AND LEAF WETNESS DURATION ON INFECTION AND DISEASE DEVELOPMENT BY *UROMYCES DIANTHI* ON CARNATIONS. M. Polek and D. M. Ferrin, Department of Plant Pathology, University of California, Riverside, CA 92521

The influence of temperature and leaf wetness duration on disease development by *Uromyces dianthi* on carnations was examined under constant temperature conditions. Eight-week-old carnation plants (cv. Improved White Sim) were inoculated with a suspension of 10^5 urediospores/ml and incubated at 10, 15, 20, 25 and 30 C for leaf wetness durations of 4, 8, 12, 16 and 24 hr. Disease was most severe on plants incubated at 15 or 20 C with a wetness duration of at least 8 hr. Disease did not develop at 10 C and developed poorly at 25 C. The latent period was 22 and 16 days at 15 and 20 C, respectively. In vitro spore germination was examined at 5, 10, 20, 25 and 30 C for incubation periods of 6, 12, 18 and 24 hr. Maximum germination (25%) occurred at 15 and 20 C after 24 hr. Germination was 11 and 9% at 10 and 25 C respectively, whereas it was 3 and 1% at 5 and 30 C, respectively.

A106

CONTROL OF GLOBODERA TABACUM SUBSP. SOLANACEARUM WITH INCREASING RATE OF FENAMIPHOS. C. S. Johnson, VPI & SU, Southern Piedmont Agricultural Experiment Station, P.O. Box 448, Blackstone, VA 23824.

Effects of fenamiphos rate (0, 1.7, 3.4, 5.0, and 6.7 kg a.i./ha) on population dynamics of Globodera tabacum subsp. solanacearum (Gts) and agronomic performance of Gts-resistant (NC 567) and susceptible (K 326) flue-cured tobacco cultivars were investigated in on-farm tests conducted in 1987 and 1988. Fewer Gts juveniles were found at higher fenamiphos rates at 2 sampling dates, but numbers of Gts cysts and eggs were not correlated with fenamiphos rate in 1987. Increased rate of fenamiphos reduced Gts cysts and eggs in 3 or 5 sampling dates in 1988. Numbers of Gts juveniles decreased with increasing rate of fenamiphos at each sampling date in 1988. Gts population densities were not significantly ($P<0.06$) lower on NC 567 than on K 326 until the final, post-harvest sampling date. Yield, gross economic returns, average prices, and flue-cured tobacco grade indices increased with fenamiphos rate in 1987, but similar trends were not statistically ($P<0.06$) significant in 1988.

A107

AN ACTION THRESHOLD FOR MANAGEMENT OF PUMPKIN POWDERY MILDEW. M. T. McGrath, Dept. of Plant Pathology, L. I. Horticultural Research Laboratory, Cornell Univ., Riverhead, N. Y. 11901-1098

A field experiment was conducted in 1989 to evaluate the feasibility of using an IPM approach for managing powdery mildew (PM) caused by Sphaerotheca fuliginea. Observed PM severity was used to time the initial application of triadimefon within a comprehensive fungicide program. Two action thresholds were evaluated: 1) a minimum of 1 out of 50 leaves with PM (AT1) and 2) an average of 1 PM colony/leaf (AT2). AT1 and AT2 occurred on day 234 and 251, respectively. Other treatments were a control, chlorothalonil, and chlorothalonil plus triadimefon. Treatments were applied 5 times. Average PM severities on both leaf surfaces were similarly low (less than 7% at maturity) for pumpkins treated with either chlorothalonil plus triadimefon or triadimefon based on AT1. PM development on lower leaf surfaces was similar to the control (about 15%) in pumpkins treated with only chlorothalonil and it was not controlled adequately with AT2. It appears that PM can be managed using AT1 in an IPM program.

A108

Soil Moisture, Genotype and Verticillium dahliae Interactions in Potato Early Dying. M. R. Cappaert, M. L. Powelson, and N. W. Christensen. Dept. of Botany and Plant Pathology, Oregon State University, Corvallis. 97331-2902.

Field microplot studies were initiated in 1989 in the Columbia Basin of Oregon to determine if soil water potential can alter the expression of resistance to potato early dying. Treatments consisted of genotypes, irrigation regimes, and inoculum levels of V. dahliae. Genotypes differed in maturity class and susceptibility to potato early dying. Three irrigation schedules ("dry", "optimum", and "wet") maintained soil moisture within the desired ranges. Plots were irrigated at 1 to 4 day intervals depending upon desired soil water potential and crop water use. Severity of PED was significantly lower ($p=0.05$) in late compared to early maturing genotypes as well as in resistant compared to susceptible genotypes. Disease severity was significantly greater ($p=0.05$) in genotypes grown in infested soil which received the "wet" irrigation schedule as compared with genotypes that received the "optimum" or "dry" irrigation schedule. Mean tuber yield was reduced 14% in the high inoculum level compared to the noninfested control across water levels regardless of maturity class or susceptibility. Irrigation regime had no effect on tuber yield.

A109

EFFECT OF TEMPERATURE AND PH ON SPORE ATTACHMENT OF FUSARIUM SOLANI F. SP. PHASEOLI ON VIGNA RADIATA IN HYDROPONIC SYSTEMS. A. C. Schuerger and D. J. Mitchell. The Land, EPCOT Center, P.O. Box 10,000, Lake Buena Vista, FL 32830, and Dept. of Plant Pathology, University of Florida, Gainesville, FL 32611.

In vitro and *in situ* experiments on the growth rate of *Fusarium solani* f. sp. *phaseoli* indicate that the optimum temperature and pH for fungal growth are between 25-28 C and 6.0-7.0, respectively. However, maximum spore attachment to plant roots by macroconidia occurs at 20 C and a pH of 4.0. Spore attachment is blocked at high and low pH (7.0 and 3.0, respectively) and at high temperature (35 C). Spore attachment to plant roots appears to be positively correlated to the secretion of an amorphous material from the terminal and foot cells of macroconidia. Observations using SEM indicate that the amorphous material is not present on macroconidia in culture nor is it present on macroconidia developing on sporodochia or individual phialides produced *in situ*.

A110

AN EVALUATION OF SEED PRIMING TECHNIQUES WITH FIVE SUGAR BEET CULTIVARS. C. M. Rush, Texas Agricultural Experiment Station, P.O. Drawer 10, Bushland, TX 79012.

A greenhouse study was conducted in which the effects of priming technique on sugar beet seedling emergence and survival in Pythium, Aphanomyces, or non-infested soil were compared. NaCl and PEG 8000 solutions were compared to a solid matrix priming technique, SMP. Washed and non-treated seed were included as controls, and five cultivars were tested. In all soils and cultivars, SMP treated seed had significantly greater emergence after three days than all other treatments. After 15 days, stands of SMP treated seed were higher in Pythium infested soil but not in control or Aphanomyces infested soil. All priming techniques resulted in significantly better emergence after 3 days than non-treated seed. Seed primed with PEG often performed no better than washed seed. Primed seed resulted in increased final stands and decreased pre-emergence damping off in Pythium infested soil. No treatment reduced seedling disease in Aphanomyces infested soil. SMP is a superior method of seed priming and the effects of priming are not cultivar specific.

A112

EFFECT OF IAA PRODUCTION ON *IN PLANTA* SURVIVAL OF *PSEUDOMONAS SAVASTANOI*. S.E. Silverstone, R.M. Bostock, D.G. Gilchrist, and T. Kosuge. Department of Plant Pathology, University of California, Davis.

Pseudomonas syringae subsp. *savastanoi* causes tumors on oleander by producing indoleacetic acid (IAA) and cytokinins. Production of IAA by the bacterium is essential for tumor formation and the genes coding for IAA production are located on the pIAA plasmid. The contributions of pIAA and IAA production to the ability of *P. savastanoi* to grow and survive in oleander leaf tissue were studied. Isogenic bacterial strains which differ only with respect to IAA production were characterized. The strains include one which lacks pIAA (pIAA$^-$; IAA$^-$), a spontaneous insertionally inactivated strain (pIAA+; IAA$^-$), and the wild-type parental strain (pIAA+; IAA+). The strains were inoculated separately or in pairs into young leaves. Growth and survival of the bacteria in host tissue were monitored over a three month period by weekly colony counts, colony hybridization, and *in situ* IAA assays. Growth rates did not differ for the 3 strains, but the wild-type strain survived longer than either mutant. The insertion mutant survived longer than the strain lacking pIAA. Coinoculation with the wild-type strain significantly increased survival of both mutant strains. These results suggest that IAA production enhances survival of *P. savastanoi* in oleander tissue and can be complemented exogenously *in planta*, and that pIAA encodes additional functions which contribute to ecological fitness.

A113

PSEUDOMONAS SYRINGAE CAN BE DISTINGUISHED AT THE PATHOVAR LEVEL IN RFLP ANALYSIS USING A RUTINASE GENE AS PROBE. M. Hendson, D.C. Hildebrand and M.N. Schroth. Department of Plant Pathology University of California, Berkeley, CA, 94720

All arginine dihydrolase negative strains of Pseudomonas, with the exception of P.s.glycinea are able to hydrolyse rutin, a glycoside constituent of many plant species (Caesar and Hildebrand, 1989). The rutinase gene of P.viridiflava F62L was cloned and used to probe EcoRI digested genomic DNA of several different Pseudomonas strains. The probe hybridized to all P. syringae, P.viridiflava and P.cichorii strains tested, as well as certain P.fluorescens and P.putida strains. RFLP patterns for P.syringae strains were identical in most cases for strains isolated from the same host and corresponding to a named pathovar. P.s.syringae strains from bean and citrus each had RFLP patterns different from P.s.syringae strains isolated from other hosts. RFLP patterns for each host group of strains appeared to be conserved and unique and may be used for the identification of fluorescent Pseudomonas strains.

A114

DIRECT MAGNETIC IMMUNOISOLATION (DMI) OF Xanthomonas campestris pv. pelargonii (Xcp). J.B. Jones and J.W.L. van Vuurde. GCREC, Univ. of Florida, Bradenton, FL 34203 and Institute for Plant Protection, P.O. Box 9060, Wageningen, The Netherlands.

In the initial development of magnetic immunoisolation (MII), difficulties existed in reducing the saprophyte to pathogen ratio. A modification, DMI, was developed in which the magnet was placed on the sample suspension surface to attract to the surface the paramagnetic iron oxide beads which have the target organism attached. In a test where washings of geranium leaves were spiked with ca. 10^3 cfu/ml of Xcp, bacterial contaminants were reduced to 156.8 and 19.6 on the agar plates, with the conventional procedure (MII) and DMI, respectively, whereas 1250 contaminants were present on the agar plate in the nontreated suspension. Recoveries of Xcp from the test suspensions were 64.9 and 47.8 percent from MII and DMI, respectively. DMI reduced contaminants significantly compared to MII, but did not significantly reduce recovery of Xcp.

A115

MOVEMENT OF GENETICALLY ENGINEERED XANTHOMONAS IN THE ENVIRONMENT. Joe Shaw[1], Fenny Dane[2], and Joe Kloepper[3]. [1]Department of Botany and Microbiology, [2]Horticulture, and [3]Plant Pathology, Auburn University, Auburn, AL 36849.

The use of genetically engineered microbes (GEMs) has a number of potential agricultural and environmental applications. Effective use of such GEMs will likely depend upon several factors including the ability to monitor the environmental fate of recombinant microbes and their exotic DNA. To address these questions we have genetically modified Xanthomonas campestris pv. campestris (Xcc) to express the bioluminescence functions of the transposon Tn4431 which carries the lux operon of Vibrio fischeri and a tetracycline resistance gene. The movement of this GEM in the environment can be monitored by bioluminescence assays, tetracycline resistance assays and PCR techniques. A limited field release of a bioluminescent strain of Xcc is planned for the spring of 1990 under the conditions of a permit issued by the USDA/APHIS. The movement of the bacteria from the inoculation site and their ability to pass Tn4431 to other bacteria will be examined.

A116

CHARACTERIZATION OF A GENOMIC REGION ENCODING XANTHOMONADIN PRODUCTION IN XANTHOMONAS CAMPESTRIS PV. CAMPESTRIS. A. R. Poplawsky. Plant Pathology/PSES, University of Idaho, Moscow ID 83843.

Yellow xanthomonadin pigments are thought to be unique to Xanthomonas spp. Their function is unknown. Sequences throughout a 26 kb clone (pIG102) from X.c.c. strain B-24 were needed to restore pigment production to thirteen pigment-negative mutants of B-24. The use of pIG102 subclones in a pigment restoration analysis divided the mutants into six restoration groups. Colony blot hybridizations showed that subclones of pIG102 were homologous to DNA from 17 pathovars of X. campestris (including pigmentless strains of pv. manihotis) but not to Pseudomonas, Erwinia, or Clavibacter. Over 130 insertions of a promoterless, lacZ gene-containing transposon (Tn3-HoHo1) have been isolated and mapped in pIG102. These insertions are being used to further define the pIG102 pigment-encoding regions.

A117

PATHOLOGICAL, RFLP AND FATTY ACID PROFILE RELATIONSHIPS BETWEEN XANTHOMONAS CAMPESTRIS FROM CITRUS AND NON-CITRUS HOSTS. J. H. Graham, J. S. Hartung, R. E. Stall, and A. R. Chase. University of Florida, CREC, Lake Alfred 33850.

Thirteen of 56 non-citrus strains produced reactions on wound-inoculated Swingle citrumelo and Duncan grapefruit leaves similar to those caused by X.c. pv. citrumelo strains from citrus bacterial spot (CBS) in Florida nurseries. Non-citrus strains of the weakly to moderately aggressive type, X.c. pv. fici, pv. maculifoliigardeniae and three strains from Strelitzia, elicited necrotic spots on spray-inoculated immature foliage and multiplied in planta as well as a weakly aggressive strain from citrus. Strains of X.c. pv. campestris, pv. phaseoli and pv. malvacearum that did not elicit necrosis failed to multiply in leaves. Most of the weakly to moderately aggressive strains of non-citrus origin could not be separated from the group of weakly aggressive citrus strains by RFLP or fatty acid profile analysis. Other X.c. strains that did not grow in planta or give a necrotic reaction were less related to the groups of citrus and non-citrus strains by these analyses.

A118

FACTORS CONTRIBUTING TO POOR POSTHARVEST DECAY CONTROL OF TABLE GRAPES. J. L. Smilanick, D. J. Henson, D. Luvisi, H. Shorey, and J. Knutson. USDA, ARS, PWA, HCRL, 2021 South Peach Avenue, Fresno, CA 93727

Insufficient box vent size or number, inadequate air movement within pallets of boxes, center-isolated boxes, and tissue-wrapped grape clusters were factors associated with poor gas penetration during commercial postharvest SO_2 fumigation of table grapes to control Botrytis cinerea. In some cases, the SO_2 concentration within packages, measured by infrared analysis, was only 10% or less that of the room atmosphere. Decay control was poor when the initial fumigation of inoculated grapes was done under warm and dry conditions compared to cold and humid conditions. More than 50% of the berries decayed after 2-3 weeks at 0°C when fumigated with 1500 ppm SO_2 at 33C and 22% RH. In contrast, only 5% berries decayed when fumigated with 300 ppm at 0C and 90% RH.

A119

EFFECT OF SINGLE VERSUS MULTIPLE RACE INOCULA ON SCREENING STRAWBERRY PLANTS FOR RESISTANCE TO COLLETOTRICHUM FRAGARIAE. Barbara J. Smith, USDA-ARS, Poplarville, MS 39470

Single and multiple inocula were compared in single inoculations and in a series of 2 or 3 inoculations to develop a technique for screening strawberry plants for resistance to several races of C. fragariae, the causal fungus of anthracnose crown rot. Conidial suspensions of 3 races were applied to 4 plants each of 4 strawberry clones in 20 treatment combinations. Disease severity (DS) was rated on a scale of 0=no symptoms to 6=plant dead. No induced resistance was found. Low DS resulted when conidia of all 3 races were combined and applied in a single inoculation. The DS of plants inoculated with a mixture of 2 or 3 races was usually close to the average DS of plants inoculated with the same races individually. High DS resulted when plants were inoculated with each of the 3 races in sequence, one every 10 days. To identify plants resistant to multiple races, inoculate with each race individually in a series of inoculations.

A120

IDENTIFICATION, OCCURRENCE, AND CRITERIA FOR INFECTION OF APPLE BY ALTERNARIA MALI. N. M. Filajdic and T. B. Sutton. Department of Plant Pathology, North Carolina State University, Raleigh, NC.

Alternaria mali, cause of Alternaria leaf blotch, was isolated from leaves of cultivar Delicious from several orchards in North Carolina in 1988. Conidial morphology was the same as reported in the literature and similar to that of a culture obtained from ATCC. AM I toxin was isolated and similar to a standard obtained from Japan. This is the first report of the disease in the US. In the summer of 1989, 60 orchards in western North Carolina were surveyed for the presence of A. mali. The pathogen was found on Delicious in all orchards; the percent infected leaves ranged from < 1% to 65%. Criteria for infection of apple seedlings were determined in vitro. Nine different temperatures (range 4-36 C), and eight different wetting durations (range 2-48 hours) were studied. The optimum temperature for infection was 24 C. Infection occurred with as little as 2 hr wetting.

A121

SURVIVAL OF VENTURIA INAEQUALIS CONIDIA AFTER DISCONTINUOUS WETTING PERIODS. C. M. Becker, and T. J. Burr. Department of Plant Pathology, Cornell University, NYSAES, Geneva, NY 14456.

The viability of V. inaequalis conidia on apple leaves was determined following discontinuous wetting periods. Treatments consisted of combinations of wet-dry-wet periods at 10, 15, 20, and 25 C, with the relative humidities (RH) during dry periods of either 60% or >90%. The first wet periods were 0 to 12 hrs, the dry periods ranged from 0 to 96 hrs, and the second wet periods were 0, 6, and 24 hrs in length. After the second wet period propagules were stained with fluorescein diacetate and calcofluor, then viewed with epi-fluorescent microscopy to quantify live propagules. At all temperatures and RH's, nongerminated conidia were unaffected by dry periods up to 96 hrs. The viability of germlings, with and without appressoria, was only slightly reduced by dry periods up to 24 hrs. Following dry periods of 96 hrs, the viability of germlings without appressoria was reduced up to 45%; viability was most reduced when the RH was >90% at 20 and and 25C. Germlings with appressoria were even more affected by drying.

A122

ETIOLOGY AND TRANSMISSION OF STIGMATOMYCOSIS DISEASE OF PISTACHIO IN CALIFORNIA. Themis J. Michailides and D. P. Morgan, Dept. of Plant Pathology, Univ. of Calif., Berkeley/Kearney Ag. Center, 9240 S. Riverbend Ave., Parlier, CA 93648.

Stigmatomycosis of pistachio is characterized by the smelly, rancid, and slimy appearance usually of the entire kernel. A Nematospora sp. was frequently isolated from kernels with stigmatomycosis. Incidence of stigmatomycosis ranged in orchards irrigated by drip 0.7-9.1%, by microjets 0.7-13.9%, by flooding 0.7-16.0%, and by sprinklers 0.7-29.1%. Four hemipterans, stinkbugs, Thyanta pallidovirens, Chlorochroa uhleri, C. ligata (Pentatomidae), and a leaffooted bug, Leptoglossus clypealis (Coreidae) successfully transmitted the fungus to pistachios both in laboratory and field experiments. In late August through early October, protected pistachio kernels had significantly lower levels of kernel necrosis but not of stigmatomycosis. Fungicide applications did not affect levels of stigmatomycosis. Although insecticide sprays reduced levels of kernel necrosis, their effect on stigmatomycosis was not clear.

A123

RELATIONSHIP BETWEEN XYLEM WOUND RESPONSE IN GRAPEVINES AND SUSCEPTIBILITY TO EUTYPA LATA. G. Munkvold and J.J. Marois, Dept. of Plant Path., Univ. of CA, Davis, 95616

Grapevines pruned in the fall have been shown to be more susceptible to Eutypa lata than those pruned in the spring. A decline in susceptibility of vines pruned in early March correlated with a rapid increase in the amount of lignin and suberin in the wounded xylem detected by the lignin-thioglycolic acid (LTGA) assay. A majority of wounds inoculated 1 day after pruning became infected; only 2% of wounds inoculated 23 days after pruning became infected. Mean LTGA/mg xylem tissue increased fourfold between 1 and 23 days after pruning. Conversely, vines pruned in late November had a very low rate of lignin accumulation. Significant differences in lignin content among cultivars exist. Different rates of lignification among cultivars and pruning dates could account for different levels and durations of susceptibility.

A124

POWDERY MILDEW OF CRANBERRY: A FIRST REPORT. S. N. Jeffers, M. J. Drilias, and P. G. Sanderson. Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Powdery mildew of cranberry (*Vaccinium macrocarpon*), although noted once previously (Anon. 1960. USDA Agric. Handbook 165), has never been described. The disease was observed first in mid-summer 1989 on greenhouse-grown, potted plants (cv. Searles) that previously had been placed in a commercial cranberry field for several days. Since then, it has been maintained on plants (cv. Stevens) growing indoors and has been transmitted to four additional cultivars -- Ben Lear, Pilgrim, Crowley, and Bain McFarlin -- by shaking infected plants with conidia over healthy plants. Powdery mildew has not been observed on plants in field locations. Mycelium and conidia of the pathogen formed on both upper and lower leaf surfaces and on succulent stem portions. Cleistothecia were produced on the lower leaf surface and stems. Each cleistothecium contained multiple asci and had long appendages that were dichotomously branched at the tips with the ultimate branches recurved. The taxonomic status of this powdery mildew fungus is presently under investigation. It clearly belongs to the genus *Microsphaera* but has taxonomic characters similar to both *M. penicillata* and *M. vaccinii* (= *M. penicillata* var. *vaccinii*).

A126

VARIANCE IN ESTIMATES OF ASCOSPORE MATURITY AND DISCHARGE IN *VENTURIA INAEQUALIS*. David M. Gadoury, D. A. Rosenberger, J. Barnard, Cornell University, NYSAES, Geneva, NY 14456, and W.E. MacHardy, University of New Hampshire, Durham, NH 03824.

Crushed pseudothecia of *V. inaequalis* have been examined routinely to assess ascospore maturity and discharge, and to aid in timing fungicide sprays for control of apple scab for over 40 years. However, the reproducibility of these assessments has not been addressed. We obtained estimates of the principal sources of variation and error in assessments by computing the variance of: (1) ratings of a standard slide set of pseudothecia by several observers, (2) ratings of the slide set by single individuals on different occasions, (3) ascospore maturity and discharge on individual leaves, and (4) ascospore maturity and discharge among different leaves. Counts from different observers were about 4 times as variable as repeated counts by a single observer. Maturity of pseudothecia was more variable between leaves than within individual leaves. The lowest variance for a given sample size was obtained by selecting one pseudothecium per leaf. Optimal sampling plans, involving the examination of 4-20 pseudothecia, were designed for early, mid, and late spring assessments of ascospore maturity and discharge based upon desired precision of the estimate.

A127

INOCULATION OF STRAWBERRY ROOTS BY COLLETOTRICHUM FRAGARIAE AND GLOMERELLA CINGULATA DOES NOT CAUSE CROWN ROT. C. M. Howard, E. E. Albregts, and C. K. Chandler, Univ. of Fla., AREC, Dover, FL 33527.

Roots of potted Chandler and Pajaro strawberry plants were inoculated in June by placing 0.5 ml of suspensions containing 3 million spores/ml of C. fragariae or G. cingulata on injured or noninjured roots at 2 points around the outside of the soil ball of each plant or by placing sections of roots infected by either pathogen in contact with noninjured roots. The plants were observed for 5 months. Natural foliar infection by G. cingulata occurred in late July and was controlled by Captan. Four of 20 control plants and 5 of 120 root-inoculated plants died from crown rot, apparently as a result of the foliar infection. G. cingulata was isolated from the crowns of 8 of the wilted plants. These results indicate that crown rot in Florida usually does not result from root infection.

A128

ISOZYME COMPARISONS OF SEPTORIA CITRI FROM AUSTRALIA AND THE UNITED STATES. M. R. Bonde, G. L. Peterson, R. W. Emmett, and J. A. Menge. USDA-ARS, Frederick, MD 21701; Dept. of Agric. and Rural Affairs, Sunraysia Hort. Res. Inst., Australia; and Univ. of CA, Riverside 92521-0122.

An embargo exists for fresh citrus fruit entering the U.S. from Australia due to reports made decades ago that species of Septoria exist on citrus in Australia not present in the U.S. However, there may be only one species, S. citri Pass., with considerable morphological variation, in the two countries. We compared isozymes of 28 isolates of S. citri (18 Australian and 10 U.S.) for 25 enzymes. Except for one Australian isolate (SHC 335), the isolates were essentially identical with an average coefficient of similarity (CS) of 0.97 and no variation for 23 of the 25 enzymes. The average CS comparing SHC 335 to other isolates was 0.58. The data supports the belief that, except for the aberrant isolate SHC 335, these isolates are of the same species.

A129

THE EFFECTS OF POSTHARVEST CALCIUM-FUNGICIDE COMBINATIONS ON CONTROL OF APPLE STORAGE DISEASES. H. E. Moline, USDA-ARS, Horticultural Crops Quality Lab, PQDI, Beltsville, MD 20705

Preliminary tests demonstrated that Ca and fungicides may interact to protect fruit from postharvest pathogens. This study was conducted to test Ca-fungicide combinations on harvested apples. Fungicides were applied at 10-50% of recommended rates; $CaCl_2$ was added at 2 or 4% (W/V). Fruit were wound inoculated, dried, and dipped in fungicide or Ca-fungicide solutions, dried, and stored at 20C. Decay ratings were made 6-12 days after inoculation. Pathogens studied included Botrytis cinerea, Glomerella cingulata, and Penicillium expansum. Fungicides tested were Captan, Benomyl, and Imazalil. Ca-fungicide enhanced decay control over $CaCl_2$ or fungicide application alone at concentrations as low as 10% of the recommended rate. Benomyl and Imazalil + Ca gave slightly better control than Captan + Ca at all reduced rates. This study demonstrates that postharvest Ca-fungicide treatment can reduce decay and may reduce fungicide residues on fruit.

A130

ROOT ROT OF YOUNG CITRUS CAUSED BY A SPECIES OF THE GANODERMA LUCIDUM COMPLEX. M.Skaria, G.S. Smith, and R. L. Gilbertson, Texas A&I Uni. Citrus Center, Weslaco, TX 78596; IPM, 45 Agri. Bldg, Univ. of Missouri, Columbia, 65211; Plant Pathology Dept, Univ. of Arizona, Agri-Bldg # 36, Tucson, AZ 85721.

In 1989, the first author reported on a disease of young citrus in Texas that was associated with a species of the G. lucidum (W. Curt:.Fr) Karst. complex. Since then, we have confirmed the Koch's postulates on two out of three different citrus root stocks. Five 1-year-old sour orange seedlings in a green house were inoculated by placing the Ganoderma basidiocarps next to the stem. Thirty-six Cleopatra mandarin seedlings grown on germination paper were surface sterilized, planted in 50 ml flasks that contained the Ganoderma isolate 89-38A, and grown at 25C. Eight young grapefruit trees, four on sour orange and four on Swingle citrumelo rootstocks, were inoculated with another isolate of Ganoderma from citrus. To date, the Cleopatra mandarin and Swingle citrumelo rootstocks have developed root rot and Ganoderma spp. was reisolated.

A131

CHARACTERISTICS OF FIVE PHENETIC GROUPS OF LEUCOCYTOSPORA FROM PRUNUS AND MALUS. R. Surve[1], G.C. Adams[1], A. Jones[1], and T. Proffer[2]. [1]Dept. of Botany & Plant Pathology, Michigan State University, East Lansing, MI 48824 and [2]Division of Plant Industry, Gainesville, FL 32602.

Isolates of Leucocytospora spp. from perennial cankers on stone and pome fruits were characterized into four groups based on colony growth, morphology and temperature optima. Allozyme polymorphisms at eleven loci separated them into five phenetic groups using cellulose acetate gel electrophoresis. Two phenetic groups had cultural characteristics similar to Leucostoma persoonii. One was found exclusively on peach, whereas the other was widespread on six Prunus spp. Three groups had Leucostoma cincta sexual states, and of these one was found exclusively on apricot and another exclusively on apple. The third occurred on several Prunus spp. Temperature optima and growth rate of the L. cincta group on apple differed from the other groups.

A132 Withdrawn

A133

SUPPRESSION OF FUSARIUM AND BACTERIAL WILT OF TOMATO FOLLOWING HERBICIDE TREATMENT. R. Cohen[1], R.A. Brammal[2], D.A. Cuppels[1] and G. Lazarovits[1], [1]Agriculture Canada, London Research Centre, 1400 Western Road, London, Ontario, N6G 2V4 and [2]Hort. Res. Inst. Ont., Hort. Exp. Station, P.O. Box 587, Simcoe, Ontario, N3Y 4N5.

The use of sublethal amounts of herbicide, applied as a seed treatment was tested as means of protecting tomato plants against the wilt pathogen Fusarium oxysporum f. sp. lycopersici. Seeds soaked for 6 and 24 h in 5 μg/ml of dinitramine (DN), gave rise to plants that were completely free of wilt symptoms after all control plants had died. Minimal impairment of growth performance of plants was observed. DN treatment also reduced symptoms caused by Pseudomonas solanacearum by 60-80%, thereby extending the usefulness of this procedure. The efficacy of chemical treatment for inducing resistance and the activity of the enzyme peroxidase was found to be correlated. This may provide a useful marker for rapid determination of the efficacy of chemicals that induce resistance. Seed treatment offers a more controllable and environmentally safer method of applying chemicals that act by inducing plant disease resistance than those previously tested.

A134

REDUCTION OF FUSARIUM ROOT ROT OF WHEAT BY CALCIUM CHLORIDE IN A GROWTH CHAMBER. G. W. Buchenau and S. S. A. Rizvi. Plant Science Department, South Dakota State University, Brookings, SD 57007.

When chloride was varied in nutrient solutions to irrigate spring wheat seedlings in washed sand in a growth chamber, emergence of cv "Butte" but not "Guard" was increased by chloride concentration of 60 mg/L in uninfested sand, but there was no effect of chloride on emergence in sand infested with Fusarium graminearum (FG). The percentage of necrotic roots in infested sand was reduced by 60 mg/L chloride on "Butte" and a similar trend occurred on "Guard". FG caused reduction in total root length of 80% on "Butte" and 48% on "Guard".

A135

SUB-LETHAL EFFECTS OF PROPICONAZOLE ON BEAN RUST. J. P. Hill, H. F. Schwartz, M. Salgado, and R. E. Farrera. Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523.

Two rows of non-treated pinto cultivar U.I. 114 plants were inoculated with a mixture of Uromyces appendiculatus var. appendiculatus urediospores at the Colorado State University Bay Farm Research Facility in 1988 and 1989. Urediospores were collected from commercial dry bean fields in eastern Colorado. Two-row plots, 1.85 m long, of pinto bean cultivar U.I. 114 or Olathe were treated with 0, 5, 10, 25, 50, or 100 g a.i./A of the systemic fungicide Tilt (propiconazole) 42, 58, and 70 days after planting in 1988 and 65 and 80 days after planting in 1989. Average pustule size, pustules per cm^2 of leaflet, and yield were recorded. Data indicate that sub-lethal fungicide application and the genetic resistance of Olathe reduced infection efficiency. Fungicide treatment rates were correlated with yield of U.I. 114 (susceptible) but had no effect on pustule size on either cultivar.

A136

REDUCTION OF THREE DISEASES IN PEANUT CAUSED BY SCLEROTIUM ROLFSII, RHIZOCTONIA SOLANI, AND CYLINDROCLADIUM CROTALARIAE WITH DINICONIZOLE. T.A. Kucharek and F.M. Shokes, University of Florida, Gainesville 32611 and Quincy, 32351.

Southern stem blight (SSB), caused by S. rolfsii, limb rot (LR), caused by R. solani, and cylindrocaldium black rot (CBR), caused by C. crotalariae, were reduced significantly (P=0.05) in peanut (Arachis hypogaea) by foliar sprays of diniconizole in field tests. Control of SSB occurred with three mid-season sprays with 0.28 kg a.i./ha/application (broadcast-equivalent rate). Higher rates or more frequent applications increased control of SSB up to 100%, but vines were stunted and yields were reduced. With 0.28 kg a.i./ha in each of three mid-season sprays, CBR was reduced by 45% (P=0.05) in the cultivar NC10C and by 33% in Florunner. With 0.14 kg/ha a.i. in 4, 5, 6, or 7 applications, LR was reduced by at least 89% (P=0.05) without excessive plant growth regulator effects. Radial growth of isolates of S. rolfsii, R. solani, and C. crotalariae was reduced, respectively, by 100, 89, and 47% on acidified PDA amended with 5 ppm of diniconizole.

A137

FUNGICIDE PENETRATION AND RETENTION IN THE RICE CANOPY. D.E. Groth, Rice Research Station, La. Agri. Exp. Stn., L.S.U. Agricultural Center, P.O. Box 1429, Crowley, LA 70527-1429.

Rice plots were sprayed with a mixture of benomyl fungicide and Dayglo dye No. A-15-N at 1.1 kg/ha each. Sprays were made at the panicle 2 mm, booting, and heading growth stages and when the canopy was wet from dew or dry. Plants were allowed to dry and were evaluated in the laboratory under a UV light for dye distribution. The upper, middle, and lower third of the plant were rated for concentration of dye using a 0 to 9 rating scale where 0 indicated no dye and 9 indicated heavy dye coverage. More fungicide/dye was deposited in the lower canopy at the panicle 2 mm stage than at the boot or heading stages. Leaf wetness reduced lower canopy deposition at the panicle 2 mm stage but increased deposition at heading. Water droplets running off of the plant were the probable cause of fungicide loss at the early growth stage. Leaf wetness apparently increased redistribution to the lower foliage in the more closed canopy at heading.

A138

SENSITIVITY OF GRAPE POWDERY MILDEW ISOLATES FROM CALIFORNIA TOWARDS FENARIMOL, MYCLOBUTANIL AND TRIADIMEFON. D. G. Ouimette and W. D. Gubler. University of California, Davis 95616

Using an excised leaf disc assay, the sensitivity of grape powdery mildew [Uncinula necator Schw. Burr.] isolates to the DMI fungicides fenarimol, myclobutanil and triadimefon was determined. Mildew isolates from vineyards where little or no DMI fungicides were used rarely had EC_{50} values towards fenarimol and myclobutanil exceeding 1 µg/ml or 5 µg/ml for triadimefon. In contrast, isolates from vineyards where a loss of efficacy towards triadimefon had occurred or where DMI fungicides were frequently applied showed reduced sensitivity towards both triadimefon and myclobutanil, and to a lesser degree fenarimol. This suggests that in vineyards with heavy mildew pressure where DMI fungicides are frequently used, a shift in the powdery mildew population towards reduced sensitivity can occur and that there is a potential for cross-resistance among the different DMI fungicides.

A139

ALGA BLIGHT OF POUTERIA SAPOTA CAUSED BY CEPHALEUROS VIRESCENS. R. T. McMillan, Jr., University of Florida, IFAS, Tropical Research and Education Center, Homestead, FL 33031

Alga blight caused by Cephaleuros virescens Kunze was observed on Pouteria sapota (Jacq.) Moore & Stearn in south Florida in 1988. Lesions on twigs and branches appear circular and light brown in color. Lesions are covered with a thin gray-green velvety layer during most of the year. The lesions expand in the rainy season and turn brick red in color due to the presence of fruiting bodies. Affected bark becomes cracked and scaly and the infected branches are stunted with sparse foliage. Cephaleurus virescens is easily controlled by good cultural practices; water, fertilizer, prunning and 2 sprays of copper at 1 pound per 100 gallons.

A140

DISEASE ENHANCEMENT AND TURFGRASS QUALITY AS INFLUENCED BY FUNGICIDES. P. H. Dernoeden and M. S. McIntosh, Dept. of Agronomy, University of Maryland, College Park, MD 20742

The main objectives of this field study were to identify turf quality and non-target disease enhancement effects of five fungicides applied to four Kentucky bluegrass, KBG (Poa pratensis) and two perennial ryegrass, PRG (Lolium perenne) cultivars. Fungicides were applied monthly from Apr through Sept between 1983 and 1987. Fungicides improved the summer quality of PRG by keeping low levels of Rhizoctonia solani in abeyance, and they did not enhance any disease in PRG. Quality of KBG was improved significantly. Most notably, triadimefon improved quality by controlling stripe smut (Ustilago striiformis) in 'Merion'; and summer patch (Magnaporthe poae) in Merion and 'Sydsport'. Chlorothalonil and thiram were associated with a significant increase in smut in Merion, and chlorothalonil increased summer patch in Merion, Sydsport, and Vantage. Triadimefon and benomyl increased leaf spot (Drechslera poae) in 'South Dakota' KBG. Iprodione did not exacerbate any disease, provided long residual leaf spot control, and elicited a color enhancement.

A141

REMISSION OF DWARFING SYMPTOMS IN SOUTHERN HARDWOODS TREATED WITH BENZIMIDAZOLE FUNGICIDES. E.P. Van Arsdel, Professional Tree Service, Inc., 2112 Cavitt, Bryan, Tx. 77801.

Benomyl, Topsin-M, or thiabendazole were applied to 7 species of oaks, 4 elms, persimmon, sweetgum, 4 ashes, and 18 other native hardwood species in treatments of declining ornamental hardwood trees. Isolation tests have shown their xylems to be inhabited with Cephalosporium diospyri (=Phyalophora obscura). Positive isolations were made from 33 of 35 species. Post treatment isolation tests showed a 95% reduction in the number of cultures obtained from xylem chips of treated trees. Treated trees developed larger, greener leaves, and longer new shoots. Cross inoculation tests produced dwarfing in 11 species. 5 other species have been inoculated and produced symptoms. Isolation tests from Louisiana, Mississippi, Florida, and Texas indicate a wide range of C. diospyri along the gulf coast. Since the fungicide treatment and the resultant decrease in C. diospyri populations increases leaf size to those found in the northern forms of several tree species, and since the large leafed northern forms are found in isolated cooler sites such as on Magazine Mountain in Arkansas, it maybe that the C. diospyri inhabiting these trees is causing their dwarfing as well as declines and diebacks.

A142

Control of Necrotic Ring Spot on Kentucky Bluegrass in Colorado, D. C. Voltz, W. Brown, Jr., and E. Milus

Isolations of fungi associated with patch diseases in Colorado since 1987 have shown that Necrotic Ring Spot, Leptosphaeria korrea is the predominant patch disease in Kentucky Bluegrass. Evaluations of selected fungicides and an organic top dressing were made in conjunction with an enhanced cultural disease control program on a naturally infected necrotic ringspot site of predominantly Kentucky Bluegrass turf. Each plot (3 m x 5.1 m) except for the undisturbed control, received an enhanced cultural practice program which included weed control and core aeration. Fungicides fenarimol (Rubigan) and diniconazol (Spotless) provided a significant reduction in number of rings and percentage of diseased turf. No other treatment significantly affected disease incidence or intensity.

A143

INFLUENCE OF FUNGICIDES ON POPULATION DYNAMICS OF *PHYTOPHTHORA PARASITICA* CAUSING ROOT ROT AND WILT OF *VINCA ROSEA*. D. M. Ferrin and R. G. Rohde, Department of Plant Pathology, University of California, Riverside, CA 92521

Soil populations of *Phytophthora parasitica* were monitored in container-grown *Vinca rosea* either not treated or treated with labelled rates and frequencies of metalaxyl (Subdue) or fosetyl-aluminum (Aliette) in separate experiments. Metalaxyl was most effective in reducing populations when applied at 1 or 2 oz./100 gal. every 4 wk. When applied at 0.5 or 2 oz./100 gal. every 4 or 8 wk, respectively, populations were generally less than for nontreated plants, but were not reduced significantly. Fresh root weights were not significantly different for treatments applied every 4 wk, but they were significantly greater than with no treatment or treatment every 8 wk. Fosetyl-Al was more effective in reducing populations when applied as a soil drench as opposed to a foliar spray. Sprayed plants supported pathogen populations equal to or greater than nontreated plants. Fresh root weights were not significantly different for any of the fosetyl-Al treatments.

A144

ASSESSMENT OF HUMAN HEALTH RISK FROM PESTICIDE RESIDUES. J. R. Tomerlin, TAS Inc., 1000 Potomac St. NW, Washington, DC 20007.

The Environmental Protection Agency (EPA) regulates the magnitude of pesticide residues remaining in or on crops.

As part of the regulatory decision making procedure, estimates of exposure are calculated as the product of mean food consumption estimates and estimates of the pesticide residue. The exposure estimate is then compared to a reference dose, a measure of toxicological significance determined from animal studies. In the absence of good estimates of residues in food as eaten, particularly for processed or cooked foods, EPA calculates worst case exposure estimates, thereby overestimating exposure and consequent human health risk. Such exposure assessments are part of EPA's normal registration, special review, and reregistration procedures. Pesticide residue data from processing studies, cooking studies, storage degradation studies, and commodity surveys in stores may yield exposure estimates which more closely approximate actual exposure.

A145

PROGRAM FOR ANALYZING ECONOMIC IMPACT OF PESTICIDE CANCELLATION. **J.E. Bailey and H.D. Tilmon. North Carolina State University, Raleigh, NC 27695-7616 and University of Delaware, Newark, DE 19717-1303.**

Public concerns over pesticide safety have increased the rate at which reevaluation and removal of pesticides have occurred. University agriculturalists are increasingly called upon to supply information on pesticides such as types and amounts used, alternative materials, and economic consequences resulting from the loss of these materials. A computer model was developed to analyze the economic impact of sequential deletion of all pesticides used for a particular crop/pest combination. The model was designed to utilize data furnished by agricultural experts who work with the crops and pests in question. Issues addressed are; 1) likely deletion order of alternatives, 2) quality and yield changes resulting from the use of these compounds, and 3) the likely proportion in which the alternative chemicals (or cultural practices) would be substituted or not used at all. This program can be used to standardize the approach used by different individuals involved in a risk/benefit study, and exposes the logic used in the analysis for future criticism and improvement.

A146

EFFECTS OF WATER STRESS ON CHESTNUT BLIGHT CAUSED BY *CRYPHONECTRIA PARASITICA*. S. J. Gao and L. Shain. Dept. of Plant Pathology, University of Kentucky, Lexington, KY 40546-0091.

Mycelial growth and conidial germination of virulent(V) and cytoplasmic hypovirulent(CH) strains of *C. parasitica* were monitored on corn meal agar media osmotically adjusted with NaCl, KCl, sucrose and a salt mixture of NaCl:KCl:Na_2SO_4 at 5:3:2. Inhibition of mycelial growth generally was not found on a sucrose or KCl induced osmotic potential above -2.0 MPa. Strains containing CH agent HV_1 were more sensitive to sodium than V strains or those strains containing CH agent HI_2. Conidia were less sensitive than mycelium to an osmotic potential of -6.0 MPa induced by sucrose or KCl. Conidia from CH strains with agents HV_1 and HI_2 were more sensitive to sodium than conidia from their isogenic V strains. The osmotic potential of American chestnut bark measured monthly during the year was between -0.8 and -2.1MPa. Excised stem segments of American chestnut were inoculated with mycelium and agar after water stress was induced by incubation of stems in polyethylene glycol or in chambers with reduced relative humidity. Canker expansion was significantly greater on those stems with increased water stress. These results indicate that the impact of water stress is greater on the host than on the pathogen.

A147

INVESTIGATION OF GENETIC RELATEDNESS AMONG dsRNAs ASSOCIATED WITH *CRYPHONECTRIA PARASITICA* ISOLATES FROM WEST VIRGINIA. S.A. Enebak[1], B.I. Hillman[2], W.L. MacDonald[1] and P.J. Bedker[2]. [1]West Virginia University. Division of Plant & Soil Sciences, Morgantown, WV 26506 and [2]Rutgers University. Cook College, Department of Plant Pathology, New Brunswick, NJ 08903.

Worldwide, double-stranded (ds) RNAs responsible for hypovirulence in the chestnut blight fungus *Cryphonectria parasitica* (syn. *Endothia parasitica*), are interrelated to varying degrees. To study the interrelationships of dsRNA found in West Virginia, recombinant cDNA libraries were constructed in the plasmid pUC8 using dsRNAs from two different isolates. The first isolate, designated D^2, has two bands of approximately 1.5 and 11 kbp. The second isolate, designated C-18, contains 11 bands ranging in size from 1 to 5 kbp. Recombinant pUC8 plasmids extracted from ampicillin resistant colonies that were white on X-gal plates contained inserts of up to 3 kbp. Two plasmids from each library were ^{32}P-labeled and used to probe dsRNA preparations from these and 3 other dsRNAs of European and North American origin. In each case, the recombinant plasmid used as a probe hybridized only to its own template dsRNA, indicating that D^2 and C-18 dsRNAs neither have close affinities to one another, or to the other dsRNAs tested.

A148

THE USE OF PROPICONAZOLE FOR CONTROL OF OAK WILT IN LIVE OAK. D. N. Appel, Department of Plant Pathology and Microbiology, Texas Agricultural Experiment Station, College Station, TX 77843.

The sterol-inhibiting fungicide propiconazole was evaluated in live oak for control of oak wilt caused by *Ceratocystis fagacearum*. Mycelial growth of the fungus *in vitro* was inhibited by 0.1 g/ml A.I. using a paper disc plate method. Wilt development was suppressed 100% in 2 to 3-yr-old containerized live oaks injected with 1.0-5.0 ml of 250 µg/ml A.I. followed by inoculation with pathogen conidia. Since June, 1987, mature live oaks at high risk to natural infection distributed throughout Central Texas were injected with propiconazole. In the first plot injected, eight untreated trees progressed from an average 0% to 58% crown loss with typical oak wilt symptoms over 24 months. However, eight trees treated preventatively at rates of 100-500 µg/ml and 1 L/in dbh progressed from 0 to 3% crown loss; no symptom of oak wilt developed. Similar results were observed in more recent plots, involving a total of 99 trees. More modest levels of disease suppression were observed in trees injected therapeutically.

A149

PLANTING SINGLE OR MULTILINE FAMILIES OF LOBLOLLY OR SLASH PINES ON SITES WITH A HISTORY OF FUSIFORM RUST. C. H. Walkinshaw. USDA, Forest Service, Southern Forest Experiment Station, Rt. 3 Box 1249-A, Asheville, NC 28806.

A number of commercial forest managers in the South have chosen to plant single families of loblolly (Pinus taeda L.) or slash (P. elliottii Engelm. var. elliottii) pine in blocks of 20 to 50 acres. These families generally have superior growth and better than average resistance to fusiform rust. But planting single families might encourage pathogen changes on sites with abundant oaks and climate favorable to the fungus. However, most resistant pine families appear to cope with alterations in the pathogen population. These families maintain their resistance on different field sites and when challenged with a variety of fungal isolates in the greenhouse. Planting multilines (many families) appears best when single families show significant genotype x environment or genotype x fungus isolate interactions.

A150

AGROBACTERIUM MEDIATED TRANSFORMATION OF POPULUS X EURAMERICANA 'OGY' USING THE PROTEINASE INHIBITOR II GENE (PIN 2) TO INCREASE PEST RESISTANCE. S. A. Heuchelin, H. S. McNabb, Jr., N. B. Klopfenstein, and R. W. Thornburg. Depts. of Plant Pathology and Forestry, Iowa State University, Ames, IA 50011.

Attempts to increase pest resistance of Populus X euramericana 'Ogy' by transformation with the Proteinase Inhibitor II (P.I. II) gene (pin 2) are in progress. P.I. II is specific for trypsin and chymotrypsin. Transformants were obtained using an Agrobacterium binary vector system with a disarmed Ti plasmid and plasmid pRT104 containing pin 2 regulated by a 35S promoter, and a selectable gene encoding neomycin phosphotransferase II (NPT II). Putative transformed shoots from co-cultured leaves were selected using 3 kanamycin selections. To date, 10 plantlets in soil-mix show putative NPT-II expression. An additional 13 are in the third selection. DNA and immunoassays (Southern and Western blots, and ELISA) to test pin 2 integration and expression are in progress. Preliminary ELISA results suggest pin 2 expression in at least one transformant. Bioassays for pest resistance will be conducted after verification of pin 2.

A151

SIMULATION OF THE DYNAMICS AND IMPACT OF ROOT DISEASES IN CONIFEROUS FORESTS OF WESTERN NORTH AMERICA. Charles G. Shaw, III, USDA Forest Service, Fort Collins, CO 80526.

Effects of pathogenic Armillaria spp. or Phellinus weirii on stand growth and development are represented by the Western Root Disease Model. This model can be used to predict effects of root disease and associated bark beetles over the next several decades and evaluate effects of a wide assortment of silvicultural practices, including stump removal for disease control. The model operates through a keyword system that allows users to modify assumptions to better represent local conditions. A critical assumption in the model is that a tree can only become an inoculum source if its root system was at least partially colonized by pathogenic Armillaria spp. or P. weirii prior to death. The model was developed through a series of workshops designed to elicit information about the disease process from many experts on the biology of the root diseases and their hosts and from forest land managers knowledgeable about the resource values being affected. The model is being adapted to simulate effects of annosus root disease.

A152

EPIDEMIOLOGY AND CONTROL OF CYCLANEUSMA MINUS NEEDLECAST OF SCOTCH PINE IN MICHIGAN. G.C. Adams. Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

Cumulative infection of Scotch pine by C. minus following three years of fungicide treatment on ten different application schedules was compared with the number of high quality trees harvested per plot. Infection was reduced significantly in all three complements of needles when 4 or 5 annual applications of a flowable formulation of chlorothalonil were used at 8-week intervals. However, disease control did not increase the quantity of high grade trees at harvest nor decrease the amount of needles cast in fall. A dispersible granular formulation of chlorothalonil did not reduce infection. Monthly spore discharge varied yearly. Discharge was correlated with occurrence of daily precipitation, but not with quantity of daily rainfall, nor with cumulative monthly rainfall. No significant differences in infection were evident among 20 seed sources of Pinus sylvestris when current-year needles were evaluated, but differences appeared in infection incidence of one- and two-year-old needles.

A153

SUDDEN DEATH OF EUCALYPTUS GLOBULUS. A. H. McCain and L. R. Costello. Department of Plant Pathology, University of California, Berkeley, CA 94720.

Sudden death is descriptive of a disease of Eucalyptus globulus that occurs in the greater San Francisco Bay area of California. The disease is "spotty" in that not every tree in a group is affected. Large trees (dbh 0.5-2 m) that appear normal in the spring are dead by the end of summer. The bark appears normal from the outside. The xylem just beneath the bark of affected trees is dead in broad, brown streaks. The discolored tissue extends into the phloem. In trunk cross sections, the discoloration is barely visible in the xylem and does not extend inward into older tissue. An unidentified fungus is readily isolated in pure culture from the discolored tissue. There is no recovery as occurs with some canker diseases. As far as can be determined from the literature, this is a new disease of E. globulus.

A154

DOGWOOD ANTHRACNOSE FUNGUS, DISCULA SP., ISOLATED FROM NECROTIC AND SYMPTOMLESS DOGWOOD FRUITS AND SEEDS. Kerry O. Britton, U.S.D.A. Forest Service, SEFES, Green St., Athens, GA 30602

Dogwood fruits were collected in Sept. from trees with symptoms of anthracnose in 11 locations in SW and NW North Carolina, and were sub-divided into four symptom types. The fleshy pulp was removed from 25 seeds in each group. All fruits and seeds were surface-sterilized in 10% bleach:10% ethanol, placed on acidified potato dextrose agar, and incubated 2 wks. Discula sp. was isolated from 2% of symptomless fruit, 2% of shrivelled red fruit, 8% of the fruit with necrotic lesions, and 12% of the entirely necrotic, shrivelled fruit. With the pulp removed, isolation percentages were similar for seed samples in the first three symptom categories. However, Discula sp. was isolated from 49% of the seeds in the entirely necrotic category, compared with only 12% of similar, unstripped fruit. Thus, isolations from whole fruits may underestimate the incidence of Discula infection, and infected seeds may provide a dispersal mechanism for dogwood anthracnose.

A155

NEW DISEASES OF FOREST TREES IN ISRAEL. *R. I. Bruck,* Z. Solel, and I. S. Ben-Ze'ev, Department of Plant Pathology, North Carolina State University, Box 7616, Raleigh, NC 27695 and ARO - Volcani Center, P.O. Box 6, Bet Dagan, Israel.

Thirty-seven new fungal diseases were found on 9 different tree species in Israel including Aleppo Pine, Italian Cypress, 3 species of Juniper, 2 species of oak, Carob and Cedar of Lebanon. All pathogens were isolated from symptomatic tissues in the field, pure cultures were established, and trees grown under greenhouse conditions inoculated with recovered fungi. The tenets of Koch's postulates were accomplished on all suspected pathogens. A partial list new diseases include: *Cupressus sempervirens*: *Diplodia pinea f.sp. cupressi*, *Seridium cardinale*, *Botryodiplodia theobromae*, *Fusarium oxysporum*; *Pinus halepensis*: *Alternaria alternata*, *Diplodia pinea*, *Fusarium oxysporum*, *Botryodiplodia theobromae*; *Juniperus oxycedrus* & *Juniperus conferta*: *Phomopsis junipivora*, *Fusarium solani*, *Pestalotiopsis funerea*, *Stigmina juniperina*, *Quercus ithaburensis* & *Quercus calliprinos*: *Choanephora* sp., *Cladosporium* sp., and *Alternaria alternata*.

A156

STEM CANKER OF PLANTATION BLACK WALNUT IN FIVE CENTRAL STATES. J. E. Cummings Carlson, M. E. Mielke, and J. G. O'Brien. Wisconsin Department of Natural Resources, 3911 Fish Hatchery Road, Madison, WI, 53711, and USDA Forest Service, State and Private Forestry, 1992 Folwell Ave., St. Paul, MN, 55108.

A survey was conducted in 1989 to determine the incidence of stem canker of plantation grown black walnut, its association with Fusarium spp., and its relationship to site and silvicultural practices in Illinois, Iowa, Minnesota, Missouri, and Wisconsin. Diseased tissue was cultured to determine the Fusarium spp. associated with cankers. Site characteristics and silvicultural practices were noted. Walnut canker was present in 84% of the 183 plantations surveyed. Regionwide, 10% of trees surveyed had walnut canker. Within plantations, disease incidence ranged from 0% to 74%. Fusarium solani was most frequently isolated throughout the five state region. Regionwide, 6.8% of the trees surveyed on upland sites had canker versus 13.3% on bottomland sites. Thirty percent of the cankers were associated with a wound and pruning wounds accounted for 48% of the wound associated cankers.

A157

RESISTANCE OF MONTEREY PINE (PINUS RADIATA) TO PITCH CANKER CAUSED BY FUSARIUM SUBGLUTINANS. M. E. Schultz, T. R. Gordon, and A. H. McCain. Department of Plant Pathology, Berkeley, CA 94720.

Five populations and 4 interpopulation crosses of Monterey pine were tested for susceptibility to pitch canker. Each population (and population cross) was represented by a minimum of 11 different clones. Each clone was represented by 2 ramits, originally separated in 1981. One set of ramits was potted and maintained in juvenile condition; the second set was allowed to mature by outplanting. Plants were inoculated with a spore suspension containing ca. 25 conidia of F. subglutinans placed in a small wound. Few lesions formed on ramits from 3 of the populations. Most of the inoculations on ramits of the other 2 populations resulted in lesions. Ramits from crosses between resistant and susceptible populations were intermediate in susceptibility. More lesions formed on potted than on outplanted ramits. These data suggest that the populations of both planted and native Monterey pine vary considerably in their susceptibility to pitch canker.

A158

RELATIONSHIP BETWEEN IN VITRO ASCOSPORE AND TOXIC METABOLITE BIOASSAYS OF POPULUS TREMULOIDES TISSUE CULTURE PLANTLETS. B.M. Kruger and P.D. Manion, SUNY College of Environmental Science and Forestry. 13210.

Two in vitro bioassays, utilizing ascospores and toxic metabolites, have been used to screen P. tremuloides for resistance to Hypoxylon mammatum. Twelve clones in tissue culture were used to test the hypothesis that sensitivity to toxic metabolites is correlated with susceptibility to ascospore inoculation. Ascospores germinated equally well on plantlets from all clones but subsequent hyphal growth and invasion was influenced by moisture stress and host genotype. The same clones were tested with toxic metabolites from three H. mammatum isolates. Significant differences were detected in clonal response to both bioassays however, sensitivity to one bioassay was not correlated with sensitivity to the other. This suggests that susceptibility of P. tremuloides to ascospore infection is not dependent on sensitivity to pathogen toxins.

A159

PURIFICATION OF IRON-CHELATING COMPOUNDS FROM GLOEOPHYLLUM TRABEUM. J. Jellison, V. Chandhoke and R. Bushway. 202 Deering Hall, University of Maine, Orono, ME 04469.

Iron plays an important role in the metabolic functions of fungi that cause wood deterioration. Gloeophyllum trabeum is able to produce low molecular weight iron-chelators. Production of these chelators (siderophores) can be induced by iron starvation. Purification of biological chelators from fungal cultures or from wood infected by G. trabeum has been achieved by freeze drying, ultra-filtration, XAD-4 column chromatography, ethyl acetate extraction and HPLC analysis. Developing a better understanding of the action of siderophores, their role in scavenging metals for fungal metabolism, and their possible function directly in lignocellulose degradation, will help us to better understand how wood degradation occurs.

A160

COMPOSTED PINE BARK MEDIA NATURALLY SUPPRESSIVE TO PYTHIUM ROOT ROTS. Y. Inbar and Harry A. J. Hoitink, Dept. of Plant Pathology, Ohio State Univ., Wooster, OH 44691

Freshly milled pine bark amended with ammonia nitrogen (0.6 kg/m^3) was composted 10-12 wk in windrows at a moisture content of 60-73% (wet weight) using process temperatures <68 C. Plug mixes (particles <0.65 cm diam) and potting mixes (particles <1.3 cm diam) were prepared by blending compost with various amounts of sphagnum peat, perlite, vermiculite and mineral additives. Highest levels of natural suppression to Pythium damping-off and root rots were induced and plant dry weight produced was highest in media amended with 25-50% (v/v) compost if media contained 40-50% moisture (w/w) and were incubated >4 days before planting. Benomyl did not reduce suppressiveness in media containing >40% moisture (w/w). The suppressive effect lasted through 4 mo in pot crops such as poinsettia and begonia. Batches of media naturally suppressive to Pythium over a 2-yr period varied in suppressiveness to Rhizoctonia.

A161

PARASITIC FITNESS OF BENZIMIDAZOLE AND DICARBOXIMIDE RESISTANT ISOLATES OF BOTRYTIS CINEREA, B. ELLIPTICA AND B. TULIPAE. T. Hsiang and G. A Chastagner. Washington State University - Puyallup. Puyallup, WA 98371.

The parasitic fitness of sensitive isolates of Botrytis cinerea (BC), B. elliptica (BE) and B. tulipae (BT) was compared to single and multiple fungicide resistant ones. Lesion development on tulip (BC & BT) and lily (BE), and sporulation for BC and BT were assessed by inoculation on detached leaves. For BC, lesion size averaged 1.5 cm^2 after 5 days, and there were no differences (P=0.05) between the fungicide groupings. Sporulation of BC isolates resistant to both benzimidazole and dicarboximide (512 spores/mm^2) was half that of sensitive or benzimidazole resistant ones. For sensitive isolates of BT, both lesion size (2.7 cm^2) and sporulation (180 spores/mm^2) were greater (P=0.05) than benzimidazole resistant isolates (2.5 cm^2 and 88 spores/mm^2). For BE, lesion length was greater (P=0.05) for sensitive isolates (9.4 mm), than benzimidazole (6.5 mm), dicarboximide (6.0 mm), or multiple resistant ones (6.6 mm).

A162

ACTIVITY OF CERTAIN OILS AGAINST FOLIAR FUNGAL PATHOGENS. J. C. Locke, USDA, ARS, Florist and Nursery Crops Laboratory, BARC-W, Beltsville, Maryland 20705.

Although petroleum oils have been used for over 100 years for their pesticidal benefits, it has only been in recent years that they have received renewed attention. Current refining technology has resulted in highly refined products with good plant safety, even for use on actively growing plants both outdoors and in the greenhouse. More recently, plant-derived oils, such as neem, have made their debut as "soft" pesticides. Evaluation of certain of these oils as possible components for IPM systems has demonstrated efficacy against some foliar fungal pathogens. Results to date suggest that common diseases such as powdery mildew, rust, and gray mold can be controlled without the use of traditional synthetic fungicides. The protectant activity of these oils has been achieved with aqueous applications containing as low as 0.25 to 2.0% (w/w) oil content.

A163

HEAT INDUCED ABNORMALITIES IN THE FLOWERS OF THE MARBLE CULTIVARS OF CHRYSANTHEMUM MORIFOLIUM. R. H. Lawson and M. M. Dienelt, USDA-ARS, Florist and Nursery Crops Lab., Beltsville, MD. 20705

Members of the Marble group of Chrysanthemum morifolium may show flower abnormalities that have been attributed to a mycoplasmalike organism (MLO). The normal inflorescence is composed of a central disk with perfect tubular florets, a border of female ligulate ray florets and an involucre of green bract phyllaries. Marble flowers develop green bracts and ligulate florets among disk florets when plants are grown at 32/26.5 C light/dark during a short day inductive period but not at 21/15.5 C light/dark at the same daylength. The control cultivar Vero developed ligulate disk florets but not disk bracts at the higher temperatures. Green disk bracts were structurally indistinguishable from involucre phyllaries by light microscopy. No MLO's were observed in phloem or other tissues in green disk bracts, involucre phyllaries, or tubular disk florets. Vascular and mesophyll parenchyma, glandular trichomes and epidermal cells contained vesicles and electron dense beaded filamentous structures that could be traced to normal cellular components.

A164

Biology of the dogwood anthracnose fungus, *Discula* spp.: 1. Effect(s) of light on pathogen growth and asexual reproduction. Brown, D.A., M.T. Windham, & R.N. Trigiano, University of Tennessee, Knoxville, TN 37901.

Growth and (asexual) reproduction rates of ten *Discula* isolates were determined in constant light (cLT) and constant dark (cDK). A complete defined medium (CM) was used in all studies. Growth increases (determined by colony dry weight) as great as 37% (range = 0-37%) in cultures grown in cLT as compared to cDK were observed. Isolates differ in their response to light with respect to growth rates, and 3 have been identified for which light does not appear to be a strong regulator of growth *in vitro*. Growth enhancement in cLT is observed in both solid and liquid CM. Cultures grown in cLT (1.5% agar CM) sporulate profusely. To date, sporulation has not been observed following the incubation of cultures in cDK.

A165

A NEW AZALEA FLOWER BLIGHT CAUSED BY A PYCNIDIAL COELOMYCETE. G. E. Holcomb. Dept. of Plant Pathology & Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State Univ. Agricultural Center, Baton Rouge 70803.

A new flower blight disease of azaleas was observed on the fall-flowering varieties Pink Camellia and Fashion at the LSU Burden and Hammond Research Stations in October 1989. Symptoms of the new disease were similar to those caused by Ovulinia azalea (tan flower discoloration and death), except that rotted tissue was firm instead of mushy and pycnidia formed on blighted flowers. A fungus belonging to the pycnidium-forming Coelomycetes was consistently isolated from infected flowers. Pathogenicity tests were positive and the same fungus was reisolated from inoculated flowers. The fungus appears to be a species of Macrophoma and efforts are continuing toward a positive identification. Fungicide tests on detached flowers indicate that Bayleton, Dithane M-45 and Chipco 26019 show promise as effective controls.

A166

THE SUSCEPTIBILITY OF FINE FESCUES TO ISOLATES OF MAGNAPORTHE POAE AND GAEUMANNOMYCES INCRUSTANS. M. L. Kemp, B. B. Clarke, and C. R. Funk, Rutgers University, New Brunswick, NJ 08903.

Magnaporthe poae and Gaeumannomyces incrustans are root-infecting fungi recently associated with patch diseases of some turfgrasses. The susceptibility of fine fescues (Festuca spp.) to isolates of these pathogens is under investigation. M. poae and G. incrustans were isolated from hard fescue (F. longifolia) turfs exhibiting summer patch symptoms in central New Jersey. A fine fescue field trial inoculated with two isolates of M. poae in June 1989 developed summer patch symptoms seven weeks later. Strong creeping red fescues (F. rubra rubra) showed significantly better resistance to these isolates than hard fescues, slender creeping red fescues (F. rubra litoralis), and Chewings fescues (F. rubra). Preliminary laboratory studies indicate that G. incrustans may also be pathogenic to species of fine fescue. Results of laboratory and field inoculations of the 1989 National fine fescue test with isolates of M. poae and G. incrustans will be reported.

A167

PATHOGENICITY STUDIES OF FUNGI ASSOCIATED WITH BERMUDAGRASS DECLINE. M. L. Elliott, University of Florida - IFAS, Fort Lauderdale Research and Ed. Center, Fort Lauderdale, FL 33314

Pathogenicity studies were conducted in a plant growth chamber with five Gaeumannomyces graminis var. graminis (Ggg) isolates, six G. incrustans (Gi) isolates and seven Phialophora sp. (Ph) isolates. 'Tifgreen' bermudagrass and 'Palmer' ryegrass were used as hosts. The growth medium was either horticultural grade vermiculite or a top soil mix of 80% sand/20% peat moss. All of the Gi isolates were pathogenic on ryegrass in both media; none demonstrated pathogenicity on bermudagrass. Three Ggg isolates were pathogenic on ryegrass and bermudagrass in both growth media. However, there were fewer symptomatic roots found on bermudagrass than ryegrass. None of the Ph isolates appeared to be pathogenic to either grass. All of the Ggg and Gi isolates were recovered from infected plant tissue to fulfill Koch's postulates. Most of the Ph isolates were also recovered from root tissue indicating they were capable of colonizing the roots.

A168

PYTHIUM SPECIES IDENTIFIED FROM TURFGRASSES IN NORTH CAROLINA. Z. G. Abad and L. T. Lucas. Plant Pathology Department, North Carolina State University, Raleigh, NC 27695-7616.

Fifteen species of Pythium were identified from 65 isolates from bentgrass, bermudagrass, centipedegrass, and tall fescue with root rot and blight during 1989 in North Carolina. Homothallic species included P. vanterpoolii (13 isolates), P. graminicola (10), P. torulosum (6), P. rostratum (4), P. irregulare (3), P. myriotylum (3), P. aphanidermatum (2), P. volutum (2), and 1 isolate each of P. aristosporum, P. oligandrum, P. paroecandrum, and P. tardicrescens. Heterothallic isolates included P. catenulatum (6), P. intermedia (5) and P. carolinianum. Of 7 unidentified species, 5 were homothallic and 2 were heterothallic. P. intermedia was isolated twice in association with P. volutum and once with P. rostratum. Because differences in sensitivity to fungicides have been observed among these Pythium species in related research, a pictorial key and procedures for rapid identification was developed. Determination of pathogenicity and fungicide sensitivity of these species is in progress.

A169

INFLUENCE OF RELATIVE HUMIDITY ON STEM BLIGHT OF GERANIUM. M. K. Hausbeck and S. P. Pennypacker, Dept. of Plant Pathology, The Penn. State University, University Park, PA 16802.

All stems of geranium (Pelargonium x hortorum) stock plants inoculated with Botrytis cinerea and incubated in a dew chamber within 12 hr of excising cuttings (stem wounding) became blighted. Disease incidence decreased to 38 and 29% when plants were inoculated 24 hr and 3 days, respectively, following stem wounding and placement in an environment of <60% relative humidity (RH) prior to incubation in a dew chamber. The area under the incidence of stem blight disease progress curve (AUDPC) revealed that a minimum of 24 hr in an environment of <60% RH between stem wounding and subsequent inoculation and incubation in a dew chamber significantly reduced blight incidence. Blight occurred in a minimum average of 94% of the stems when plants were placed in an environment of <60% RH for 24 hr following inoculation, prior to incubation in a dew chamber. When plants were inoculated and subjected to <60% RH for 3, 5, and 7 days prior to incubation in a dew chamber, an average of 69, 85, and 50% of the stems, respectively, became blighted. According to AUDPC data, a minimum of 24 hr in an environment of <60% RH following inoculation significantly reduced stem blight incidence.

A170

RESIDUAL ACTIVITY OF FUNGICIDES APPLIED TO GERANIUMS IN THE GREENHOUSE. G. W. Moorman and R. J. Lease, The Pennsylvania State University, Department of Plant Pathology, 211 Buckhout Laboratory, University Park, PA 16802.

To use mixtures of fungicides for the management of fungicide resistant fungal populations, the chemicals must have residual activities similar to that of the chemical which is 'at risk' to the development of resistance and must effectively control the target organism. Fungicides were applied singly at the recommended rate to seed geraniums (Pelargonium X hortorum cv. Red Elite) in a greenhouse. The day after treatment and weekly for 3 wks, 1 cm diameter disks were excised from leaves. The disks were inoculated with Botrytis cinerea spores suspended in 0.1M dextrose and were then incubated for 10 da at 20C in 16 hr light/8 hr dark. Then, the number of infected disks was recorded. Control, as compared to disease on inoculated fungicide-free tissue, was calculated. Control provided by the fungicides was as follows: vinclozolin (the 'at risk' chemical) provided 100% control initially, and 60% by wk 3; chlorothalonil, 83% initially, 75% at wk 3; mancozeb, 53% to 40% for all 3 wks; cupric hydroxide, 38% initially, 15% at 3 wks; zineb, 43% initially, 15% by wk 3; and dichloran, 10% initially, 3% by wk 3.

A171

IDENTIFICATION OF LEPTOSPHAERIA KORRAE WITH CLONED DNA PROBES. N. Tisserat, S. Hulbert, and A. Nus. Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Differentiation of Leptosphaeria korrae from other ectotrophic fungi associated with patch-type diseases of turfgrasses can be difficult because of similarities in colony morphology and the inability to induce ascocarp formation in some isolates. A more reliable means of identification was developed using two different cloned DNA fragments from L. korrae. The probes, 0.8 and 1.2 Kb in size, were specific to L. korrae, and did not hybridize to genomic DNA of other patch-disease causing fungi, including L. narmari, Gaeumannomyces incrustans, G. graminis vars. graminis, avenae, and tritici, Magnaporthe poae, Ophiosphaerella herpotricha, and Rhizoctonia solani. The DNA probes did not cross-hybridize, but hybridization patterns from EcoRI digests of genomic L. korrae DNA implied that they belonged to the same repetitive element family. No polymorphisms were detected between L. korrae isolates with either probe.

A172

SERIOLOGICAL AND ULTRASTRUCTURAL ANALYSIS OF A ROD-SHAPED VIRUS IN A ST. AUGUSTINEGRASS LINE IMPORTED INTO THE UNITED STATES FROM AFRICA. C. Edward McClelen, R. W. Toler, and *D. R. Huff. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Tx 77843, *B-Four Corporation Research Center, Box 321, West Columbia, Tx 77846.

In 1963, Stenotaphrum secundatum (Walt.) PI 291594 was introduced into the United States from Zimbabwe and has been maintained in Georgia and more recently in Texas. PI 291594 was found to be infected by a rod-shaped virus with a modal length of 712 nm and a width of 12 nm. The maximum length found was 781 nm, and 70% of the particles were between 678 and 753 nm. Immunosorbent electron microscopy (ISEM) using sugar cane mosaic virus-strain E antiserum from the American Type Culture Center showed trace reactions to infected PI 291594 and strong cross-reactivity to maize dwarf mosaic virus-B infected sorghum. ISEM using SCMV-D, and H antisera gave negative reactions with infected PI 291594. Thin sections of PI 291594 contained amorphous viral inclusions in epidermal cells.

A173

PRODUCTION EFFECTS OF APPLE MOSAIC VIRUS ON *ROSA DILECTA*. C. L. Palmer, R. K. Horst, and R. H. Langhans. Departments of Plant Pathology and Floriculture, Cornell University, Ithaca, NY 14853.

Rosa dilecta cultivars were bud graft-inoculated with leaf pieces infected with apple mosaic virus (ApMV). After two years each plot of twelve plants contained between one and six ELISA-tested positive plants with foliar symptoms. Flowers from symptomatic and nonsymptomatic plants were cut and graded per plot based on number, mean height and weight during a 25 week period. Compared to nonsymptomatic ELISA-tested negative plants in the same plots, symptomatic plants had a mean reduction overall of 32% in rose cuts: Bridal Pink 25%, Gold Rush 43%, Lavande 49%, Royalty 31%, Samantha 7%, Sonia 38%. The low percentage of cuts in Samantha suggests a varietal difference in tolerance to ApMV. Although no dramatic decrease in height or weight occurred, the severe reduction in flower cut due to virus infection indicates the need for ApMV-clean commercial operations.

A174

ELECTROPHORETIC KARYOTYPES OF AFLATOXIN PRODUCING AND NON-PRODUCING ASPERGILLUS SPP. N. P. Keller, USDA, ARS, Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA 70124.

Chromosomes of the aflatoxin producing fungi Aspergillus flavus and A. parasiticus were separated using contour-clamped homogeneous electric field (CHEF) gel electrophoresis. Linkage group studies have indicated that A. flavus has eight chromosomes (Papa, Can. J. Microbiol. 30:68-73, 1984). CHEF electrophoresis showed that the chromosomes are grouped in two size ranges: one group of 4+ chromosomes of approximately 3-5 megabases and one group of 1-2 chromosomes larger than the S. pombe 7.0 megabase chromosome. Although similar, the chromosome of A. flavus and A. parasiticus are not identical in size. Karyotype profiles of A. flavus isolates suggest chromosome translocations have occurred in some strains. Chromosome separation conditions and chromosome profile comparisons among and between related Aspergillus spp. are discussed.

A175

CHARACTERIZATION OF INF56, A GENE EXPRESSED DURING INFECTION STRUCTURE DEVELOPMENT OF THE BEAN RUST FUNGUS. X.-L. Xuei, S. Bhairi, R.C.Staples and O.C.Yoder. Dept. of Plant Pathology and Boyce Thompson Institute, Cornell Univ., Ithaca, NY 14853.

Uredospores of the bean rust fungus, Uromyces appendiculatus, differentiate to form infection structures in response to the topography of stomatal guard cells or to 0.5um ridges on a plastic surface. Twenty differentiation-specific clones have been isolated by cascade-hybridization. They were divided into six classes based on cross hybridization. One of the clones, 13kb in size, encoded several transcripts, including a predominant one of 1.0kb. The gene specifying the 1.0kb transcript (INF56) was subcloned on a 2.5kb fragment, sequenced, and the open-reading frame was determined. It contains a 67bp intron and encodes for a 23kD polypeptide as determined by in vitro translation. There is more than one copy of INF56 in the genome. No homology to DNA from other plant pathogenic fungi has been found. Large quantities of the INF56 specific protein can be obtained in E. coli. Experiments on the immunochemical localization of the gene product are in progress.

A176

CLONING AND PRELIMINARY CHARACTERIZATION OF AN ENDO-POLYGALACTURONASE GENE FROM COCHLIOBOLUS CARBONUM. J. S. Scott-Craig and J. D. Walton, MSU/DOE Plant Research Laboratory, Michigan State University, East Lansing, Michigan, 48824.

Purified endo-polygalacturonase (PG) from Cochliobolus carbonum was digested with trypsin and both intact enzyme and purified fragments were sequenced by Edman degradation. Degenerate oligonucleotides were synthesized based on the amino acid sequence data and were used to prime DNA synthesis from genomic DNA using the polymerase chain reaction. An 800bp PCR product was used as a probe to identify genomic and cDNA clones of the PG gene. Northern analysis indicates that the gene encodes a 1.3 kilobase mRNA which is present at high levels when the fungus is grown on pectin and at very low levels when the fungus is grown on sucrose. The DNA sequence and intron structure of the gene have been determined and deletion derivatives have been constructed and re-introduced into C. carbonum by transformation.

A177

CHROMOSOME-LENGTH POLYMORPHISMS AMONG FOURTEEN RACES OF USTILAGO HORDEI: CHARACTERIZATION AND MEIOTIC SEGREGATION. K. McCluskey and D. Mills, Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, 97331-2902.

Chromosome-length polymorphisms were identified among seventeen strains of Ustilago hordei, the causal agent of covered smut of barley. Fourteen of the strains represent different races; the three additional strains complete a meiotic tetrad of one race. A chromosome-length polymorphism segregated 2:2 among the members of this tetrad. Several other tetrads have been examined, and all have conserved karyotypes. Among the strains representing the fourteen races, chromosomes varied in size from 170 to 3,500 kilobases, and in number from 16 to 21. Homologous chromosomes which vary in size by up to one hundred kb among the different strains have been identified by Southern hybridization. Random homologous DNA probes have allowed distinction between two chromosomes that are nearly identical in size. Conserved genes from other fungi are being used to identify linkage groups. Novel techniques for chromosome sample preparation without the need for protoplast formation have been developed.

A178

TRANSFORMATION OF AN ECTOMYCORRHIZAL FUNGUS. V. Barrett, J.Shaw, P.A. Lemke. Department of Botany and Microbiology, Auburn University, Auburn, Alabama 36849.

Transformation of the ectomycorrhizal basidiomycete, Laccaria laccata, has been based on selection for resistance to hygromycin B using a plasmid with the Aspergillus nidulans gpd promoter and the Escherichia coli hpt structural gene (Barrett et al. 1990 Appl. Microbiol. Biotechnol. in press). The observation that promoters and structural genes function in heterologous fungi implies considerable similarity for mechanisms of gene expression. We have begun experiments to assess promoter efficiency in L. laccata. Using protoplasts, we have transformed L. laccata with sequences coding for several heterologous promoters and are currently exploring the use of these promoters in conjunction with the reporter genes B-galactosidase and luciferase. Quantitation of gene expression from known promoters will provide the basis for cloning homologous L. laccata sequences with promoter activity. Such sequences will allow efficient expression of introduced genes in the transformed fungus.

A179

CHARACTERIZATION OF CIRCULAR PLASMIDS FOUND IN THREE SPECIES OF Pythium. Frank N. Martin, Plant Pathology Department, University of Florida, Gainesville, Fla 32611

Circular plasmids have been identified in two isolates of P. aphanidermatum and one isolate each of P. torulosum and an unidentified echinulate isolate. Plasmids were multimeric, with unit lengths ranging from 3.36 to 4.94 kb and were unique for each isolate based on restriction maps of cloned plasmids and Southern hybridization analysis. Hybridization studies revealed no sequence similarity between the plasmids and the nuclear or mitochondrial genome of the isolates from which they were recovered. For two isolates, Southern transfers of double digests of total DNA and hybridization with cloned plasmids provided plasmid DNA fragments which did not correspond to the sizes expected from the restriction map or from partial digests, indicating that rearrangements of the plasmid had occurred. Investigations to determine if the plasmids are of mitochondrial origin are in progress.

A180

DETECTION OF SEQUENCE HOMOLOGY BETWEEN DOUBLE-STRANDED RNA SPECIES ISOLATED FROM PHYTOPHTHORA INFESTANS. J. R. Newhouse, P. W. Tooley, and O. P. Smith. USDA-ARS, Frederick, MD 21701.

Northern-blot hybridization analysis was employed to evaluate the sequence homology between species of double-stranded (ds) RNA from Mexican, Dutch, and Peruvian isolates of Phytophthora infestans. Double-stranded RNA species extracted from two Mexican isolates (without bands in common) were prepared for use as two separate 5'-end-labeled ^{32}P probes. Hybridization was observed only between comigrating dsRNA species of the isolates from Mexico, the Netherlands, and Peru. No homology was detected between dsRNA species of the two Mexican isolates, and neither probe showed homology with a high molecular weight dsRNA band from the Dutch isolate. These results indicate that at least three non-homologous groups of dsRNA exist in P. infestans, and support the hypothesis that A_2 mating type isolates of the fungus recently found in Europe may have originated in Mexico.

A181

RFLP BASED PHYLOGENY OF FUSARIUM OXYSPORUM F. SP. LYCOPERSICI (FOL) REVEALS NO ASSOCIATION BETWEEN RACE AND GENETICALLY ISOLATED POPULATIONS. K. S. Elias, T. Katan, and D. Zamir, Volcani Ctr., Bet Dagan, Hebrew Univ. Jerusalem-Fac. Agric., and Otto Warburg Ctr., Rehovot, ISRAEL.

RFLPs were employed to estimate genetic diversity in FOL. We utilized 110 isolates of FOL previously characterized for form species, race, geographic origin, vegetative compatibility group (VCG), and isozyme electrophoretic phenotype. Fifty DNA clones from a random genomic library from one isolate of FOL, were used as probes for Southern hybridization to total genomic DNAs cut with 4 restriction enzymes. Polymorphisms were recorded, coefficients of similarity were calculated, and cluster analysis was performed. Few RFLPs were observed among isolates within a VCG whereas the majority of RFLPs occurred among isolates between VCGs. This suggests that VCGs are genetically isolated populations with differing ancestral progenitors. In addition, races could not be distinguished by RFLPs. It appears races have arisen several times, i.e. at least once within each VCG of FOL.

A182

REPETITIVE GENOMIC SEQUENCES FOR DETERMINING RELATEDNESS AND FOR DNA FINGERPRINTING OF STRAINS OF FUSARIUM OXYSPORUM. H.C. Kistler, E.A. Momol, and U. Benny. Plant Pathology Department, University of Florida, Gainesville, FL 32611

Length polymorphisms in restriction fragments containing moderately repetitive DNA sequences have been identified in the fungus Fusarium oxysporum. Arbitrarily chosen genomic clones pEY1, pEY7 and pEY10, containing, respectively, 1.1, 2.3 and 1.2 kb of fungal DNA, were used to identify the repetitive sequences. When used as a probe for hybridization to restriction endonuclease digested DNA from various strains, distinctive banding patterns were observed for each strain of the four formae speciales of F. oxysporum studied. These probes have utility for phylogenetic analysis and for DNA fingerprinting individual strains. Identification of genetic markers specific for strains will aid in epidemiological studies of the fungus.

A183

ANALYSIS OF THE dsRNA ASSOCIATED WITH NB88-58, A HYPOVIRULENT STRAIN OF THE CHESTNUT BLIGHT FUNGUS FROM NEW JERSEY. B.I.Hillman and Y. Tian, Dept. of Plant Pathology, Rutgers University, New Brunswick, NJ 08903.

NB88-58 is a hypovirulent strain of the chestnut blight fungus characterized by a single dsRNA species of approximately 13 kbp. We have made cDNA libraries representing the genome of this dsRNA beginning with oligo d(T) and random primers. 95 cDNA clones were mapped relative to one another by Southern blotting, and 45 of these were mapped in greater detail by restriction and nucleotide sequence analysis such that a contiguous map of overlapping cDNA clones was obtained. The sequences of the 5' terminal 3 kbp and 3' terminal 2 kbp (relative to the plus strand), and several internal regions were determined and compared to that of the French-derived strain EP713. The overall similarity between these two strains was approximately 50%, with some regions of notable conservation, and the two appear to have similar genetic organizations.

A184

MOLECULAR ANALYSIS OF THE dsRNA ASSOCIATED WITH EP747, AN ITALIAN HYPOVIRULENT STRAIN OF THE CHESTNUT BLIGHT FUNGUS. **M.P. Brown and B.I.Hillman, Dept. of Plant Pathology, Rutgers University, New Brunswick, NJ 08903.**

The hypovirulent strain EP747 of the chestnut blight fungus, derived from dsRNA of Italian strain EP420, is characterized by two dsRNA species, the larger of which is approximately 13 kbp. We have made cDNA libraries representing the genome of this dsRNA. We have used those cDNA clones to examine relationships between EP747 and other chestnut blight-associated dsRNAs, and between this dsRNA and the genomic DNAs of several Cryphonectria parasitica strains. The dsRNA of EP747 was more closely related to that of EP713 than to any other dsRNA tested, as determined by spot hybridization analysis. The cDNA clones tested showed no significant sequence similarity with any C. parasitica genomic DNA, including that of Flat mutants derived from EP747 and their ascospore progeny, as determined by Southern blots capable of detecting single copy genes.

A185

POPULATION STRUCTURE OF THE RICE BLAST FUNGUS IN THE PHILIPPINES. E.S. Borromeo, R. Nelson, J.M. Bonman, and H. Leung. International Rice Research Institute, P.O. Box 933, Manila, Philippines.

The genetic variability of Pyricularia oryzae in the Philippines was examined by RFLP analysis, using a 1.4 kb EcoR1 fragment that contains a repetitive DNA element. This element (probe 613) was isolated from rice-infecting isolate V86013, and differed from the repetitive element described by Hamer et al. (PNAS 86:9981). Forty-nine RFLP types were defined among 106 isolates from 17 provinces in the Philippines. Genetic diversity was calculated to be 0.95 based on Nei and Tajima's method (Genetics 97:145). RFLP types varied in frequency and distribution. Four types accounted for 40% of the samples whereas the frequencies of several other types ranged from 1 to 4%. One predominant type (13%) was distributed in 10 of 17 provinces, while another type (10%) was restricted to a single province. Preliminary analysis showed no apparent association between RFLP types and virulence phenotypes.

A186

INCREASE IN IMPORTANCE OF CUCUMBER MOSAIC VIRUS INFECTION IN GREENHOUSE-GROWN BANANAS IN MOROCCO. M. Bouhida and B.E. Lockhart, Complexe Horticole, B.P. 438, Agadir, Morocco/Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Until recently, cucumber mosaic virus (CMV) infection was of trivial importance in banana-producing areas of Morocco, where there has been rapid expansion of the crop under plastic greenhouses. The CMV strain occurring in banana produced mild mosaic symptoms and had little effect on plant growth. Within the last two years outbreaks of CMV infection have occurred, involving a strain of CMV not previously recorded in Morocco. Symptoms include severe yellow mosaic and systemic necrosis similar to heart-rot symptoms described elsewhere. Infection rates attain 100% in some greenhouses. The severe CMV strain is readily aphid-transmitted to and from banana, and has spread to weeds and vegetable crops. Intercropping of muskmelon with newly planted banana, and occurrence of high populations of Aphis gossypii exacerbate the disease. It is speculated that the severe strain of CMV was introduced recently into Morocco in infected banana planting stock.

A187

GENETIC MULTIPLE VIRUS RESISTANT BELL PEPPERS. B. VILLALóN, Texas Agricultural Experiment Station, 2415 E. Hwy 83, Weslaco, Texas 78596.

Bell peppers, one of about 20 different domesticated Capsicum annuum L. types, has for many years been the most important fresh market and home garden pepper. Increased demand for this high Vitamin C, low calorie vegetable salad item has stimulated production in Texas and other areas throughout the world. Most known commercial bell pepper cultivars are susceptible to virus diseases. The Texas Agricultural Experiment Station at Weslaco has released two new bell pepper varieties and has also developed hundreds of advanced inbred lines with resistance to tobacco etch virus, pepper mottle virus, potato virus Y, tobacco mosaic virus, tobacco ringspot virus, and cucumber mosaic virus. Improved inbred lines of large, four lobed bell type peppers are being proposed for release to the seed industry for development of hybrid or open-pollinated varieties.

A188

SELECTIVE AND DIFFERENTIAL ISOLATION OF *PHYTOPHTHORA* SPP. FROM *THEOBROMA CACAO* L. AND *CITRUS* SPP. M. L. Oliveira and J. A. Menge, Department of Plant Pathology, University of California, Riverside, CA 92521

Phytophthora which cause black pod of cacao in Brazil, *P. palmivora, P. capsici* and *P. citrophthora*, were tested for the production of pectate lyase, polygalacturonase, lipase, protease, deoxyribonuclease, phosphatase, urease, cellulase and amylase. These studies led to the development of a differential and selective medium which enabled detection of macroscopic differences among the three species of *Phytophthora*. It contained agar 15 g, peptone 5 g, beef extract 3 g, soluble starch 2 g, pimaricin 0.01 g, ampicillin 0.125 g, pentachloronitrobenzene (PCNB) 0.133 g, hymexazol 0.075 g and rifampicin 0.01 g per L H_2O and permitted reliable quantitative differential isolation of *Phytophthora* from infected tissues and naturally or artificially infested soil. It proved useful for studies involving survival and competition of the three *Phytophthora* species, and for selective isolation from other hosts such as citrus infested with more than one *Phytophthora* pathogen.

A189

DISTRIBUTION OF CITRUS TRISTEZA VIRUS IN A SWEET ORANGE GROVE CONTAINING YOUNG AND OLD TREES. J. G. Lee and J. A. Dodds, Department of Plant Pathology, University of California, Riverside, CA 92521.

DsRNA analysis and ELISA was used to determine the incidence of citrus tristeza virus (CTV) in a grove containing Navel sweet orange trees, 26 yr old Frost Nucellars alternately planted with 4 yr old Washingtons. The rootstock for both was Troyer citrange. An initial survey indicated that 100% (50/50) of old trees and 26% (13/50) of young trees were infected with CTV. Eleven of the young trees that tested positive for CTV were near the eastern edge of the grove suggesting aphid transmission into the grove from this direction and not at random from old trees. Titer of CTV was higher in old compared to young trees. Expression of a specific dsRNA (0.5×10^6) was more frequent in old trees than young trees. No other strain specific dsRNAs have been detected in samples from trees in this grove.

A190

SPECIES AND DISTRIBUTION OF ROOT-KNOT NEMATODES ASSOCIATED WITH BEAN PRODUCTION REGIONS IN COLOMBIA AND PERU. B.A. Mullin, G.S. Abawi, M.A. Pastor-Corrales and J.L. Kornegay. Dept. of Plant Pathology, Cornell Univ., Geneva, NY, USA, and Bean Program, CIAT, Cali, Colombia.

Over 100 populations of root-knot nematodes (RKN) were collected from bean (Phaseolus vulgaris) roots and soils from the major production areas of Colombia and Peru. Species were identified using perineal patterns prepared from 16 adult females of each population. Meloidogyne incognita was the most widespread and most frequently identified species, as it occurred in all the states included in the survey in both countries and was detected in 65% of the samples. M. javanica was detected in 14% of the collections and occurred in both hot and cool regions. M. hapla was detected only in cool-temperature regions and was present in 7% of the collections. M. arenaria occurred in association with other RKN species only in 4 samples. Seventeen collections consisted of a mixture of two or more of the above species.

A191

PECTINOLYTIC ACTIVITY IN XANTHOMONAS CAMPESTRIS PV. VESICATORIA. C. Beaulieu, G. V. Minsavage and R. E. Stall. Plant Pathology Department, Univ. of Florida, Gainesville, FL 32611.

Although pectinolysis is not a feature commonly associated with X. c. pv. vesicatoria (Xcv), several pectinolytic strains that cause bacterial spot of tomato and pepper were found among strains from South America. Studies were undertaken to characterize the pectinolytic activity in strain Xv 56. Most of the pectinolytic activity was due to the secretion of an unique pectate lyase. The pI of this enzyme was estimated to be 8.8. Neither pectin lyase nor polygalacturonase activity was detected. From a genomic library of DNA from Xv 56, a cosmid conferring the ability to depolymerize polypectate when transferred to a non pectinolytic strain of Xcv was identified. A 1.4 kb fragment of this cosmid hybridized to genomic DNA of other pectinolytic xanthomonads but not to genomic DNA of Erwinia chrysanthemi.

A192

RESTRICTION FRAGMENT LENGTH POLYMORPHISM AMONG STRAINS OF *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* RECOVERED FROM BEAN AND OTHER HOSTS. D.E. Legard and J.E. Hunter. Cornell University, New York St. Agri. Exper. Station, Dept. Pl. Pathology, Geneva, NY 14456

We evaluated restriction fragment length polymorphisms among 53 strains of *Pseudomonas syringae* pv. *syringae*. Twenty-nine strains were isolated from brown spot lesions on bean, nine strains were isolated from lima bean, and the rest from 10 other hosts. Southerns of DNA digested with EcoR I or Hind III were hybridized to two random probes from a cosmid library of *P. s. syringae*, and a hrp locus (pHIR11) cloned from *P. syringae*. The size of hybridizing fragments was determined and a similarity matrix computed by comparing strains on a pairwise basis for the presence or absence of fragments. Data were converted into a phenogram using an unweighted pair-group method with arithmetic mean algorithm. This analysis confirmed that *P. s. syringae* is a genetically diverse pathovar. Strains of *P. s. syringae* isolated from bean were more closely related to each other than to strains from other hosts. However, the bean strains formed two sub-groups that are only 60% related, and few strains had identical haplotypes. These data are consistent with the hypothesis that *P. s. syringae* pathogenic on bean arose from 1 or 2 founding events rather than from multiple foundings. Construction of restriction maps of the hrp region in *P. s. syringae* isolated from bean is in progress.

A193

TRANSCRIPTIONAL ACTIVITY OF FLUORESCENT SIDEROPHORE GENES FROM PSEUDOMONAS SYRINGAE IN SITU ON LEAF AND ROOT SURFACES. S.E. Lindow and J.E. Loper, Dept. Plant Pathology, UC, Berkeley, CA 94720, and USDA, ARS, HCRL, Corvallis, OR 97333.

Plasmid pJEL1701 was constructed by cloning an 8 kb DNA fragment, which is essential for fluorescent siderophore production (Flu) of P. syringae, upstream of a promoterless ice nucleation gene (inaZ) in the stable plasmid, pVSP61. P. syringae and Pseudomonas fluorescens strains harboring pJEL1701 expressed ca. 1 ice nuclei/cell when grown in a iron-deplete minimal medium, and only ca. 10^{-5} nuclei/cell when the medium was supplemented with 10^{-4} M $FeCl_3$. In contrast, cells harboring pJEL1703, comprised of an ice nucleation gene transcribed from its native iron-constitutive promoter cloned in pVSP61, expressed ca. 10^{-3} nuclei/cell in both iron-replete and iron-deplete media. On leaf surfaces, P. syringae and P. fluorescens cells harboring pJEL1701 expressed ice nucleation activity (INA) at a level ca. 3 fold less than those harboring pJEL1703. On bean root surfaces, P. fluorescens cells harboring pJEL1701 expressed INA at a level ca. 10 fold less than those harboring pJEL1703. P. fluorescens cells harboring pJEL1701 expressed less INA on root surfaces in soil amended with FeEDDHA or FeEDTA. Results suggest that the flu gene is transcribed on leaf and root surfaces, but at only ca. 5% the level observed in an iron-deplete medium.

A194

PHYSICAL AND GENETIC CHARACTERIZATION OF THE CLONED COR REGION FROM PLASMID PAC002 OF PSEUDOMONAS SYRINGAE PV. TOMATO DCT6D1. S-W. Ma[1], V.L. Morris[2], and D.A. Cuppels[1]. [1]Agriculture Canada Res. Centre and [2]Dept. of Microbiol. & Immunol., Univ. of Western Ontario, London, ON, N6G 2V4, CANADA.

Pseudomonas syringae pv. tomato, causal agent for bacterial speck of tomato, produces the chlorosis-inducing phytotoxin coronatine. In strain DC3000, genes controlling toxin production (cor region) are chromosomally-located; in strain DCT6D1, they are present on pAC002, a 100-kb plasmid. Southern blot analysis demonstrated that the cor region was plasmid-borne in 9 of the 10 pv. tomato strains examined. We have recently isolated the cor region from a pLAFR3 library of pAC002 DNA. The isolated DNA was mapped using restriction enzymes BamHI, EcoRI, and XhoI. The genetic and transcriptional organization of the plasmid-encoded cor genes was determined by insertional mutagenesis using Tn3-Spice and then compared to that of the chromosomally-located cor region of strain DC3000.

A195

CHARACTERIZATION OF A PUTATIVE REGULATORY LOCUS REQUIRED FOR ENDOPOLYGALACTURONASE PRODUCTION IN PSEUDOMONAS SOLANACEARUM. Caitilyn Allen, Merelee M. Atkinson, and Luis Sequeira. Department of Plant Pathology, University of Wisconsin-Madison, Madison WI 53706.

An extracellular polygalacturonase (PG) produced by *P. solanacearum* is believed to play a role in bacterial virulence. We have cloned the structural gene for this enzyme, pehA, and have found that it is physically linked to the vir2 cluster of hrp genes previously described by Boucher et al. A second, unlinked locus, pehR, is required for expression of the pehA polygalacturonase. When a pehR::Tn5 insertion mutant, which does not produce this PG, was complemented in trans by the wild-type region, it produced 8X the wild-type level of the enzyme. This suggests that pehR encodes a positive trans-acting regulator. A synthetic oligonucleotide derived from the A. tumefaciens virA locus hybridized to pehR indicating that pehR may be related to a family of bacterial two-component regulatory systems. To test the hypothesis, DNA sequencing of pehR and phosphorylation studies with pehR-encoded proteins are being completed.

A196

A CLONED REGION OF PLASMID pPT23A IS REQUIRED FOR CORONATINE SYNTHESIS IN PSEUDOMONAS SYRINGAE pv. TOMATO PT23.2. S. A. Young and C. L. Bender, Dept. of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947.

Plasmid pPT23A is involved in coronatine biosynthesis in P. syringae pv. tomato PT23.2. Previously, coronatine-defective (Cor^-) mutants of PT23.2 contained either Tn5 insertions or deletions in plasmid pPT23A or were missing pPT23A entirely. In the present study, a cosmid library of pPT23A was constructed in pLAFR3. A 52 kb cosmid clone, designated PSAY1, was transformed by electroporation into three classes of Cor^- mutants and two nonproducers of coronatine (E. coli K-12 and P. s. syringae PS51). Organic acids were extracted from these transformants and analyzed by reverse-phase high performance liquid chromatography and a potato disc bioassay. Cosmid pSAY1 restored coronatine production to five mutants containing either Tn5 insertions or deletions in pPT23A. However, the acquisition of pSAY1 did not confer coronatine production to a mutant lacking pPT23A or to the two nonproducers of coronatine.

A197

MOLECULAR CLONING OF CELL WALL DEGRADING ENZYMES FROM *AGROBACTERIUM TUMEFACIENS* BIOVAR 3. K.M. Ophel, D.A. Jones and A. Kerr. Department of Plant Pathology, Waite Agricultural Research Institute, Glen Osmond, South Australia. 5064.

Both tumorigenic and nontumorigenic strains of the grapevine pathogen, *Agrobacterium tumefaciens* biovar 3 (AT3), produce a root specific decay of grapevine (Burr *et al.*, 1987). All AT3 strains tested in our study have both endopolygalacturonase and endoglucanase activity. A genome library of a wild type AT3 strain was constructed in a cosmid vector and clones encoding production of both enzymes were isolated. The genes are expressed both in *E. coli* and in *Agrobacterium* biovar 1. Production of either enzyme alone does not cause root decay of grapevine. Clones were mutagenized with derivatives of the transposon Tn3 and enzyme-minus mutants have been obtained. The mutated clones have been used to construct marker exchange mutants in AT3. The AT3 mutants will be screened for activity on grape roots in order to investigate the role(s) of the enzymes in pathogenicity.

A198

USE OF PUTATIVE TRANSPOSABLE ELEMENTS AS PROBES FOR POPULATION STUDIES OF THE BACTERIAL BLIGHT PATHOGEN OF RICE. M.R. Baraoidan, R. Nelson, J.E. Leach[1], T.W. Mew, and H. Leung. International Rice Research Institute, P.O. Box 933, Manila, Philippines and [1]Kansas State Univ., Manhattan, KS 66506

The transposon trapping vector pL3Sac (Kearney and Staskawicz, 1990. J. Bacteriol. 172:143) was used to isolate putative transposable elements from Xanthomonas campestris pv. oryzae (Xco), the causal agent of bacterial blight of rice. Restriction analysis of the inserted elements showed that six transposable elements were identified from four strains of Xco tested. Each strain yielded one to four elements. One of the transposable elements, designated TnX1, was used as a probe for Southern analysis of a collection of strains of Xco in the Philippines. Nineteen RFLP types were defined among 103 strains tested. RFLP types were not specific to race groups; race 3 shared RFLP types with four other races in the Philippines. Preliminary results on the regulation of transposition in Xco are discussed.

A199

MOLECULAR CLONING OF AN ERWINIA AMYLOVORA rcsB GENE REQUIRED IN POLYSACCHARIDE SYNTHESIS. A. Mendoza and A. K. Chatterjee, Department of Plant Pathology, University of Missouri, Columbia, Missouri 65211.

The production of extracellular polysaccharide (EPS) by E. amylovora (Ea) is required in its pathogenicity on apples and pears. Our previous work with the cloned Ea rcsA gene [rcs=regulation of capsule (=EPS) synthesis] suggested that it, in conjunction with another gene product (i.e. RcsB), activates polysaccharide synthesis in E. coli. The presence of an rcsB homolog in Ea was indicated by the restoration of EPS production in the Ea strain E-8 by an E. coli rcsB but not by the Ea rcsA gene. By screening an Ea cosmid library in an E. coli rcsB strain (SG1086) we obtained colonies that were mucoid at 28-30° C in a glucose supplemented medium. The mucoid phenotype was not expressed at 37° C or in the absence of a metabolizable sugar. The cosmids complemented both rcsA and rcsB mutations in E. coli. From one of the cosmids, we have obtained subclones that complemented either the rcsB mutation or the rcsA mutation. Thus, in Ea at least two genes are required for the activation of EPS synthesis.

A200

A NEW SET OF *ERWINIA CHRYSANTHEMI* PECTIC ENZYMES PRODUCED DURING GROWTH ON PLANT MATERIAL. S. Kelemu and A. Collmer. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Erwinia chrysanthemi EC16 is known to produce a number of pectolytic enzymes which can contribute to its maceration ability. A mutant containing site-directed mutations in the genes *pehX*, *pelX*, *pelA*, *pelB*, *pelC*, and *pelE*, respectively coding for exo-poly-α-D-galacturonosidase, exo-polygalacturonate lyase, and the four known isozymes of pectate lyase retained significant maceration activity. Activity-stained isoelectric-focusing gel analysis of culture supernatants revealed that the mutant produced several new pectic enzymes when grown on plant tissue extracts or chrysanthemum cell walls, but not when grown on pectate or a variety of other carbon sources. Filter-sterilized, pectolytic, culture supernatants had pectate lyase activity in A_{230} assays and macerated plant tissues. Analysis and manipulation of DNA fragments cloned from the mutant to produce pectolytic *Escherichia coli* transformants will clarify the role of these enzymes in pathogenesis.

A201

PROTECTION AGAINST DETRIMENTAL EFFECTS OF POTYVIRUS INFECTION IN TRANSGENIC TOBACCO PLANTS EXPRESSING THE PAPAYA RINGSPOT VIRUS COAT PROTEIN GENE. K. S. Ling, S. Namba, C. Gonsalves, J. L. Slightom* and D. Gonsalves. Dept. of Plant Path., Cornell Univ., Geneva, NY 14456. *The Upjohn Company, Kalamazoo, MI 49007.

Transgenic tobacco plants expressing the papaya ringspot virus (PRV) coat protein gene were obtained by transformation with Agrobacterium tumefaciens. Strong positive reactions were obtained when leaf extracts from transgenic plants were tested with anti-PRV monoclonal antibody in ELISA. The coat protein comprised 0.2% of the total leaf soluble proteins. A protein band with similar molecular weight to that of PRV coat protein was detected in leaf extracts of ELISA-positive transgenic plants by Western blot analysis with the anti-PRV antibody. Transgenic plants with high levels of PRV coat protein expression showed significant delay in symptom expression and severity after inoculation with other potyviruses, including tobacco etch, pepper mottle, and potato virus Y.

A202

SEQUENCE OF INFECTIOUS CLONES OF TWO MECHANICALLY TRANSMISSIBLE ISOLATES OF BEAN GOLDEN MOSAIC GEMINIVIRUS. J. C. Faria[1], R. L. Gilbertson[1], F. J. Morales[2], D. R. Russell[3], P. G. Ahlquist[1], S. F. Hanson[1], and D. P. Maxwell[1]. [1]Department of Plant Pathology, University of Wisconsin, Madison, WI 53706; [2]CIAT, Cali, Colombia; [3]Agracetus, Inc., Middleton, WI 53717.

Bean golden mosaic geminivirus (BGMV) causes a serious disease of Phaseolus vulgaris in Latin America and is transmitted by Bemisia tabaci. Isolates from Brazil are not mechanically transmitted, whereas isolates from the Caribbean and Central America are mechanically transmitted. Sequence analysis of full-length clones of BGMV isolates from Guatemala (BGMV-GA) and the Dominican Republic (BGMV-DR) showed that each isolate was composed of two DNA components, designated A and B, which have four and two ORFs, respectively. Sequence comparisons indicated that BGMV-DR and BGMV-GA were closely related to a BGMV isolate from Puerto Rico but not to one from Brazil, which is more similar to tomato golden mosaic geminivirus. Beans inoculated with cloned DNAs of BGMV-GA/component A and BGMV-DR/component B or with BGMV-DR/component A and BGMV-GA/component B developed typical and attenuated symptoms, respectively, implying component asymmetry.

A203

INFECTION OF BEANS WITH CLONED GEMINIVIRAL DNA MEDIATED BY ELECTRIC DISCHARGE PARTICLE ACCELERATION. R. L. Gilbertson[1], J. C. Faria[1], S. F. Hanson[1], F. J. Morales[2], P. Ahlquist[1], D. P. Maxwell[1] and D. R. Russell[3]. [1]Department of Plant Pathology, University of Wisconsin, Madison, WI 53706; [2]CIAT, Cali, Colombia; [3]Agracetus, Inc., Middleton, WI 53717.

Geminiviruses are small plant viruses that possess a single-stranded DNA genome. Bipartite, whitefly-transmitted geminiviruses are a major constraint on bean production in the tropics. Four isolates of bean-infecting geminiviruses--bean golden mosaic virus from Brazil (BGMV-BZ), Guatemala, and the Dominican Republic and bean dwarf mosaic virus--were cloned. Full-length linear double-stranded DNA components A and B were not infectious when mechanically coinoculated onto bean primary leaves by surface abrasion but were infectious when inoculated into radicles of beans by electric discharge particle acceleration. Infection of beans by cloned DNAs of BGMV-BZ, which has never been mechanically transmitted as virions or cloned DNAs, was achieved using particle acceleration and indicated that this method circumvents plant barriers to mechanical transmission. Particle acceleration will facilitate genetic analysis of these geminiviruses and may allow for efficient introduction of viral nucleic acids or virions of other viruses into hosts that are refractory to mechanical transmission.

A204

POLYMERASE CHAIN REACTION: MOLECULAR TECHNOLOGY TO ENHANCE DETECTION AND DIAGNOSIS OF POME FRUIT VIROIDS. A. Hadidi and X. Yang, National Plant Germplasm Quarantine Laboratory, ARS, USDA, Beltsville, MD 20705.

Rapid in vitro amplification of segments of genomic DNA sequences or transcripts is now possible with very high specificity and fidelity by using Taq I DNA polymerase in a polymerase chain reaction (PCR). We have extended and modified PCR to amplify viroid concentration using cDNA synthesis in total nucleic acid extracts from apple scar skin viroid (ASSV), dapple apple viroid, or pear rusty skin viroid-infected tissue. PCR-amplified products then were analyzed by gel electrophoresis or by Southern blot hybridization with a ^{32}P-labeled ASSV cRNA probe. The new procedure is more sensitive than existing diagnostic methods and provides information about viroid detection without requiring large samples or using molecular hybridization. Our results also suggest the potential utility of the PCR technology in detecting other viroids, plant viral satellite RNAs, plant viruses, and possibly other plant pathogens.

A205

COAT GENES OF COWPEA CHLOROTIC MOTTLE VIRUS AND BROME MOSAIC VIRUS CAN BE EXCHANGED WITHOUT AFFECTING HOST SPECIFICITY OR SYSTEMIC MOVEMENT. R.F. Allison, M. Janda, C. Thompson, P. Ahlquist, Institute for Molecular Virology and Dept. of Plant Pathology, Univ. of Wisconsin, Madison, WI 53706.

Although the tripartite genomes of cowpea chlorotic mottle virus (CCMV) and brome mosaic virus (BMV) share common organization and sequence similarity, these viruses have distinct host ranges. BMV systemically infects monocotyledonous hosts, while CCMV infects only dicotyledonous plants. In vitro transcripts from full length cDNA clones of BMV or CCMV RNAs are infectious to their natural hosts. In protoplasts RNAs 1&2 of either virus replicate in the absence of RNA3; no replication, however, is detected with heterologous combinations of RNAs 1&2. Combinations of RNAs 1&2 from one virus plus the heterologous RNA3 replicate and are encapsidated in protoplasts, but similar combinations do not initiate systemic infections. Therefore, the 3a or coat proteins, encoded by dicistronic RNA3, must either singly or cooperatively influence host range. The role of the two RNA3 genes in host specificity has been assessed by exchanging individual genes between the viruses. Whole plant inoculations indicate that BMV and CCMV coat protein genes can be exchanged freely without affecting host specificity. Involvement of the 3a protein in host specificity is confirmed, since exchange of these genes abolishes systemic infections in both hosts.

A206

BOTH HELPER VIRUS AND SATELLITE RNA AFFECT SYMPTOMS OF TURNIP CRINKLE VIRUS INFECTION. C.W. Collmer and S.H. Howell, Boyce Thompson Institute, Cornell University, Tower Road, Ithaca, NY 14853

Previous studies on the three satellite RNAs associated with the isolate of turnip crinkle virus now designated TCV-JI (John Innis isolate) have identified satellite RNA C as a virulent satellite. Addition of this satellite to helper virus (TCV-JI) inoculum containing the smaller satellite RNA D results in an intensification of the normally mild symptoms in turnip. In an attempt to carefully assess the effects of the two TCV satellite RNAs on symptomatology, we tested each satellite with TCV RNA derived from a cDNA clone of TCV-B (Berkeley isolate). The much milder symptoms caused by the satellites with this virus isolate indicate a role for the helper virus in symptom intensification. Ongoing sequence analysis of the TCV-JI isolate has thus far revealed relatively few nucleotide differences with TCV-B, thus offering the opportunity to localize determinants for symptom expression in the viral genomes.

A207

TRANSFORMATION OF *DATURA INNOXIA* WITH GENE I OF PEANUT CHLOROTIC STREAK VIRUS (CAULIMOVIRUS). D. A. Ducasse, J. M. Kiernan, and R. J. Shepherd. Dept. of Plant Pathology, Univ. of Kentucky, Lexington, KY 40546.

Sequence homology of gene I of some caulimoviruses with the 30K transport protein of TMV as well as localization studies by immuno-electronmicroscopy of cauliflower mosaic virus (CaMV) gene I protein suggest that gene I of the caulimoviruses might be involved in cell-to-cell movement of virus. In order to understand the relationship between the gene I protein and intercellular transport of virus, *D. innoxia* plants were transformed with gene I of peanut chlorotic streak virus (PClSV), a member of the caulimovirus group that infects this plant systemically at high temperature. Southern blot analysis of transgenic plants indicated the genomic integration of gene I. Expression of gene I protein was confirmed by western blot analysis using an affinity purified antiserum raised against a 25 amino acid synthetic peptide corresponding to the carboxy-terminus of the protein.

A208

CLONING OF GENES ENCODING EXTRACELLULAR METALLOPROTEASES FROM *ERWINIA CHRYSANTHEMI* EC16. G. S. Dahler, F. Barras and N. T. Keen. Department of Plant Pathology, University of California, Riverside, CA 92521

A 14-kb *Bam*HI-*Eco*RI DNA fragment cloned from strain EC16 contained a gene encoding a protease inhibitor as well as three tandom *prt* genes encoding metalloproteases. The *prt* genes were separated from the inhibitor gene by a ca. 4-kb region that was necessary for extracellular secretion of the proteases. When individually subcloned downstream from vector promoters, the *prt* genes each led to substantial extracellular secretion of the proteases in *E. coli*, provided that the 'required region' was supplied *in cis* or *trans*. The *prt*C gene was sequenced and had high homology to metalloproteases previously described from *Serratia* spp. and *E. chrysanthemi* strain B374. Mutations in strain EC16 that reduced protease production did not detectably affect virulence in chrysanthemum stems.

A209

CHARACTERIZATION OF A GENE REQUIRED IN TRANSCRIPTIONAL ACTIVATION OF THE PECTIN LYASE STRUCTURAL GENE, PNLA, OF ERWINIA CAROTOVORA SUBSP. CAROTOVORA (ECC). J. L. McEvoy, A. Chatterjee and A. K. Chatterjee, University of Missouri, Columbia, Missouri 65211.

Pectin lyase (Pnl) production occurs in many strains of soft-rotting Erwinia species in response to DNA-damaging agents. This induction in ECC strain 71 requires RecA function. Complementation of regulatory mutations in ECC71 and reconstitution of an inducible Pnl system in a RecA$^+$ E. coli led to the detection of a cosmid carrying the activator gene, pnlR. By subcloning, pnlR was localized on a 6.7 kb EcoRI DNA segment. pnlR was inactivated using the mini-Mu-lac element Mu dI1734. Insertion of the element in one orientation yielded a β-galactosidase-inducible phenotype in response to the DNA-damaging agent, mitomycin C, in RecA$^+$ (LexA$^+$?) ECC and RecA$^+$ LexA$^+$ E. coli strains. These findings suggest that the stimulation of Pnl production by DNA-damaging agents results from an increased pool of the pnlR product that is required for transcription of pnlA.

A210

EXPRESSION OF THE ERWINIA CAROTOVORA SUBSP. CAROTOVORA (ECC) AEP GENE IS REQUIRED IN THE PRODUCTION OF EXTRACELLULAR PROTEINS. H. Murata and A. K. Chatterjee, Department of Plant Pathology, University of Missouri, Columbia, Missouri 65211.

In Ecc71, we identified an aep gene that is necessary for production of extracellular enzymes including pectate lyases (Pels). We subsequently constructed an aep-lacZ transcriptional fusion, and placed the DNA on the chromosome of a LacZ$^-$ strain, AC5006. The resulting Aep$^-$ strain (AC5024) failed to produce extracellular enzymes but was inducible for β-galactosidase production; induction ratios were ca. 3 and 5 when grown in presence of citrus pectin and celery extract (CE), respectively. Under these growth conditions Pel production in an Aep$^+$ strain was also stimulated. The kinetics of the induction of β-galactosidase and extracellular enzymes in AC5024 carrying an aep$^+$ plasmid revealed that a high rate of Pel, protease and cellulase production commenced after the initiation of β-galactosidase synthesis in a CE medium. Thus, aep gene expression is necessary for the production of the extracellular enzymes in Ecc71.

A211

MOLECULAR CLONING, NUCLEOTIDE SEQUENCE, AND MARKER-EXCHANGE MUTAGENESIS OF THE *PEHX* GENE OF *ERWINIA CHRYSANTHEMI* EC16. S. Y. He and A. Collmer. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

The *pehX* gene encoding extracellular exo-poly-α-D-galacturonosidase (exoPG) was isolated from a genomic library of the pectate lyase-deficient *E. chrysanthemi* mutant UM1005 (a Nal^r, Kan^r, Δ*pelABCE* derivative of EC16). The cloned *pehX* gene was expressed highly from its own promotor in *Escherichia coli*, and most of the enzyme was localized in the periplasm. The nucleotide sequence of *pehX* revealed the presence of an amino-terminal signal peptide and an open reading frame encoding a preprotein of 64,608 dalton. Enzymatic activity was retained when 10% of the C-terminus of exoPG was removed by subcloning. Analysis of the constructed mutants, CUCPB5008 (Pel$^+$, Peh$^-$) and CUCPB5009 (Pel$^-$, Peh$^-$), indicated that exoPG can contribute significantly to bacterial utilization of polygalacturonate, the pitting phenotype on pectate semi-solid agar and the induction of pectate lyase, but not to the maceration capability of the bacterium.

A212

DIFFERENTIATION OF PSEUDOMONAS SYRINGAE PV. MORSPRUNORUM FROM P. S. PV. SYRINGAE USING A DNA PROBE FROM P. S. PV. TOMATO. J. M. Paterson and A. L. Jones. Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

A DNA hybridization probe developed in Georgia for detection of Pseudomonas syringae pv. tomato (Pst) was evaluated for its ability to differentiate isolates of P. s. pv. morsprunorum (Psm) from P. s. pv. syringae (Pss) collected from cherry, prune and plum in Michigan and other geographical locations. The DNA probe was specific for isolates of Psm. Southern blots of EcoRI and PstI digested genomic DNA revealed that the DNA probe hybridized to multiple restriction fragments. Eight and twelve isolates of Psm contained a common 3.5 kilobase (kb) EcoRI and 2.7 kb PstI fragment, respectively. No restriction fragments of Pss were detected with the Pst probe. The ability of the Pst probe to detect plant pathogens in situ will be discussed.

A213

MOLECULAR ANALYSIS OF A LOCUS FROM *Pseudomonas syringae* pv. *syringae* REQUIRED FOR LESION FORMATION. E. M. Hrabak & D. K. Willis, Dept. of Plant Pathology and USDA/ARS, University of Wisconsin, Madison 53706.

The wild-type *lemA* locus of *P. syringae* pv. *syringae* B728a is required for development of typical brown spot lesions on bean (*Phaseolus vulgaris*). Mutations in this locus affect lesion development, as well as production of an extracellular protease and the toxin, syringomycin. Tn*3*-HoHo1 was used to mutagenize a 9.7 kilobase (kb) cosmid subclone carrying the intact *lemA* locus. The direction of transcription and length (<4.3 kb) of the *lemA* locus have been determined. Sequencing of this region is in progress. Because of the pleiotropic phenotype of *lemA* mutants, it is hypothesized that *lemA* may be a regulatory locus.

A214

TN*PHOA* TAGGING OF *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE HRP* GENES ENCODING POTENTIALLY EXPORTED PROTEINS. H.-C. Huang[1], S. W. Hutcheson[2] and A. Collmer[1]. [1]Department of Plant Pathology, Cornell University, Ithaca, NY 14853. [2]Department of Botany, University of Maryland, College Park, MD 20742.

Cosmid pHIR11 contains a cluster of *hrp* genes from *Pseudomonas syringae* pv. *syringae* (Pss) that enables *P. fluorescens* to elicit the hypersensitive response (HR) in tobacco leaves. pHIR11 was mutagenized with Tn*phoA* in *Escherichia coli* CC118, then mobilized by triparental mating into *P. fluorescens* and screened for loss of HR elicitation. Hrp$^-$ mutations were marker-exchanged into the genome of Pss and determined to define 11 complementation groups based on analysis of merodiploids. Two of the Pss mutants produced blue colonies on medium containing 5-bromo-4-chloro-3-indolyl-phosphate, indicating that Tn*phoA*-generated hybrids expressed alkaline phosphatase activity. Western blot analysis revealed that the hybrid proteins had molecular weights of 58 K and 61 K and were produced only in minimal medium. The results indicate that some Hrp proteins have either periplasmic domains or are exported out of the cytoplasm.

A215

FURTHER CHARACTERIZATION OF A GENE CLUSTER REQUIRED FOR EPS PRODUCTION AND VIRULENCE IN *PSEUDOMONAS SOLANACEARUM*. Douglas Cook and Luis Seuqeira, University of Wisconsin-Madison, Department of Plant Pathology, Madison, WI 53706.

A 28 kb cosmid clone that complements two EPS-/vir- Tn*5* mutants of *P. solanacearum* was characterized by saturation mutagenesis. A gene cluster of 6.5 Kb contains five complementation units required for virulence, four of which are essential for normal EPS production. Tn*3*-mediated gene fusions were used to quantify gene expression in the various complementation units by measuring B-glucuronidase activity both in planta and in broth cultures. Two classes of EPS-affected mutants could be distinguished visually based on colony morphology and chemically according to level of N-acetylgalactosamine in semi-purified EPS. All but one of the mutants grew as well as wildtype in broth culture, but in planta growth of the mutants was substantially reduced. One group of mutants produced no visible EPS, grew poorly or not at all in the plant and were weakly virulent. Another group produced an intermediate level of EPS, grew slower than wildtype in the plant and retained moderate virulence.

A216

BACTERIAL UTILIZATION OF TRANSGENIC PLANT SYNTHESIZED AND SECRETED MANNITYL OPINES. M. A. Savka and S. K. Farrand, Department of Plant Pathology, University of Illinois at Urbana-Champaign, IL 61801.

An Agrobacterium-mediated binary transformation system was constructed to introduce mannityl opine anabolic genes from an octopine-type Ti plasmid into plants. Transgenic tobacco plants were regenerated which expressed resistance to kanamycin and synthesized the mannityl opines. One regenerant examined in detail exhibited a selfed R1 segregation pattern for kanamycin resistance of 3:1. Mannityl opine biosynthesis cosegregated with kanamycin resistance at a frequency of 1.0. Analysis of R3 progeny from R2 selfings showed segregation patterns consistant with the T-DNA insert being located on a single chromosome. Genomic Southern analysis confirmed the presence of T-DNA in DNA from the transgenic plant. Transgenic plants grown autotrophically in a mineral salts solution secreted the opines from their roots into the media. Pseudomonas and Agrobacterium strains containing genes conferring catabolism of the mannityl opines could grow in mineral salts solution at the expense of the plant synthesized and secreted mannityl opines. These results show that genes 0', 1' and 2' of pTi15955 T_R-region are sufficient for the biosynthesis and secretion of the mannityl opines, that these opines are synthesized and secreted from the roots of transgenic plants grown under autotrophic conditions, and that the secreted opines can be utilized by plant-benefical bacteria.

A217

COPPER-BINDING OUTER MEMBRANE AND PERIPLASMIC PROTEINS FROM THE COPPER RESISTANCE OPERON OF *PSEUDOMONAS SYRINGAE* PV. *TOMATO*. J.-S. Cha and D. A. Cooksey. Department of Plant Pathology, University of California, Riverside, CA 92521.

P. syringae strains carrying the plasmid-borne copper resistance operon (*cop*) accumulate copper when grown in the presence of high levels of cupric sulfate. Proteins produced from the *cop* operon after copper induction were purified by cellular fractionation methods, ion exchange chromatography, and gel filtration chromatography. Purified CopA and CopC proteins bound copper, as measured by atomic absorption spectroscopy. CopA was associated with the outer membrane; CopC was periplasmic. CopB was tightly-associated with the outer membrane fraction that bound copper, but after purification from the membrane, CopB did not contain copper. Accumulation of copper in the outer membrane and periplasm by *cop* gene products, and probably other outer membrane components, may prevent the entry of toxic levels of copper ions into the cell and therefore confer resistance.

A218

CHARACTERIZATION OF *ERWINIA STEWARTII* MUTANTS UNABLE TO CAUSE WATERSOAKING SYMPTOMS ON CORN. D. L. Coplin, D. R. Majerczak, L. D. Tuttle, R. D. Frederick, D. K. Estes, and J. Costa. Dept. of Plant Pathology, The Ohio State University, Columbus OH 43210.

Cosmid pES1044 from *Erwinia stewartii* contains two large clusters of genes (*wtsA* and *wtsB*) that are needed for this bacterium to cause watersoaked lesions on corn seedlings. Chromosomal deletion mutants of the *wts* region, DM3001 and DM3020, were isolated. Tn*5*, Tn*5lac*, and Tn*3*HoHoI mutagenesis of pES1044 and complementation analysis confirmed that *wtsA* and *wtsB* are separate transcription units; a third complementation group, which was represented by a single mutation, was also identified. Initial growth of *wtsA* and *wtsB* mutants in seedlings was not impaired. DM3020, which is deleted for *wtsA* and *wtsB*, produced extracellular polysaccharide (EPS) that was similar to wild-type EPS in sugar composition and proton and carbon NMR spectra. *wts::lac* gene fusions were expressed *in planta*, and in a minimal salts-glucose-casamino acid medium during mid-log phase, but not in rich media or during other phases of growth.

A219

COMPLEMENTATION OF HRP MUTANTS OF *ERWINIA AMYLOVORA* WITH DNA OF *ERWINIA STEWARTII*. S. V. BEER, R. J. Laby, and D. L. Coplin*. Departments of Plant Pathology, Cornell University, Ithaca, NY 14853 and The Ohio State University*, Columbus, OH 43210.

Transposon induced Hrp$^-$ mutants of *E. amylovora* are not pathogenic to immature pear fruit and fail to elicit the hypersensitive response in tobacco. The cloned *hrp* gene cluster of *E. amylovora* restores the Hrp phenotype. A cosmid, pES1044, contains the *wts* (water-soaking) region of *E. stewartii* that is required for symptoms of Stewart's wilt. The cosmid hybridized with certain subclones of the *hrp* cluster of *E. amylovora*. Hrp function was restored fully by pES1044 to some of those mutants with insertions in the regions of hybridization. Hrp function was not restored by pES1044 to mutants with insertions in regions that did not hybridize with pES1044. Experiments to determine possible complementation of *E. stewartii wts* mutants with DNA of the *hrp* cluster of *E. amylovora* are in progress. The observation of interspecific complementation for pathogenicity indicates conservation of genetic and functional homology between two species of *Erwinia*.

A220

THE CULTIVAR-SPECIFIC ELICITOR ASSOCIATED WITH EXPRESSION OF AVIRULENCE GENE D FUNCTIONS IN SOYBEAN CELL CULTURE Stanley J. Tamaki*§, Diane Lawrence*, Noel Keen† and Mark Stayton*; *Department of Molecular Biology, University of Wyoming, Laramie, WY 82071; §ClearGene, Inc., University of California-Richmond Field Station, Richmond, CA 94804-4698; †Department of Plant Pathology, University of California at Riverside, Riverside, CA 92521-0122.

Expression of the pathogen avirulence gene D in Pseudomonas syringae pathovars as well as in Escherichia coli, results in the biosynthesis of a unique, low molecular-weight compound (the cultivar specific elicitor or SE). The SE induces a strong necrotic reaction on the leaves of soybean cultivars which express the dominant disease resistance gene, Rpg4. Cultivars which carry a recessive allele at this locus show no visible signs of necrosis upon inoculation. In leaf disks from soybean lines which express Rpg4, the SE inhibits the incorporation of [^{35}S]methionine into protein. Thus, we have initiated studies to determine the minimum level of tissue organization required to maintain cultivar-specific sensitivity to the SE compound. Soybean callus cultures derived from hypocotyl tissue were digested with cell wall macerating enzymes yielding mainly single cells with a small proportion of protoplasts. A 20-min pre-incubation of these cells with low concentrations of SE, inhibited [^{35}S]methionine incorporation by 70% in Rpg4 cells but only 15% in rpg4 cells. At ten-fold higher SE concentrations, [^{35}S]methionine incorporation is completely inhibited in both genotypes. Thus, at appropriate SE levels, soybean cell cultures show a cultivar-specific response to the SE.

A221

XANTHOMONAS CAMPESTRIS PV. TRANSLUCENS GENES DETERMINING HOST SPECIFIC VIRULENCE AND GENERAL VIRULENCE ON SMALL GRAINS. V.R. Waney and D.W. Gabriel. Plant Pathology Dept., Univ. of Florida, Gainesville, FL 32611.

X.c. pv. translucens strain Xt-216 causes leaf streak on barley, wheat, oats, rye, and triticale. Three thousand Tn5 Gus insertional derivatives of strain Xt-216 were inoculated on the five hosts. Prototrophic insertional derivatives affected in virulence on one of the hosts but not affected on the rest (host specific virulence, Hsv$^-$) were identified at frequencies of 0.07% (Barley$^-$), 0.04% (Wheat$^-$), 0.07% (Oats$^-$), 0.10% (Rye$^-$) and 0.07% (Triticale$^-$). Prototrophic mutants affected in virulence on all homologous hosts (Vir$^-$), and failing to give a hypersensitive response on tobacco and cotton were obtained at a frequency of 0.42%. The cloned mutated region from one Vir$^-$ derivative hybridized to the cloned hrp locus (pVir2) from Pseudomonas solanacearum (C.A. Boucher et al., 1986. J. Bacteriol. 169:5626-32). Complementation of several Vir$^-$ and Hsv$^-$ mutants with DNA fragments from Xt-216 was achieved. Based on Tn5 insertional analysis, two loci may be required for virulence of Xt-216 on barley. Growth in planta, and analysis of DNA fragments which complement host specific virulence mutants will be discussed.

A222

IDENTIFICATION AND PARTIAL CHARACTERIZATION OF PLASMIDS PRESENT IN THE BEET LEAFHOPPER TRANSMITTED VIRESCENCE AGENT. M. E. Shaw, B. C. Kirkpatrick, and *D. A. Golino. Department of Plant Pathology, University of California, and *USDA/ARS, Davis, CA 95616.

Southern blot analyses, using cesium chloride purified supercoiled DNA as probe, identified at least two plasmids in plants and Circulifer tennelus leafhoppers infected with a type strain (FC-83-13) of the beet leafhopper transmitted virescence agent (BLTVA-MLO). No plasmids were detected in healthy hosts. The plasmids were approximately 18 and 3 kbp in size. Southern blot analyses, using native and cloned BLTVA plasmid fragments as probes, showed the BLTVA plasmids shared no homology with the plasmids that are present in strains of the western aster yellows MLO. The 18 kbp BLTVA plasmid contains a single EcoR1 site which should facilitate cloning full-length copies of this plasmid in lambda EMBL-3.

A224

SEROLOGICAL DIFFERENTIATION OF MAIZE DWARF MOSAIC VIRUS (MDMV) STRAINS A, B, D, E, F AND O. S. L. Lenardon[1], D. T. Gordon[1], and R. E. Gingery[2], [1]Dept. of Plant Pathology, The Ohio State Univ.; [2]USDA-ARS, Wooster, OH 44691.

Polyclonal antisera (PcAs) to MDMV strains A, B, D, E, F and O, collected 1 wk after a single injection with intact virions (As-1wk), reacted with homologous capsid protein (HmCP), but not with homologous capsid core protein (HmCCP) in western blots. CCPs were obtained from intact virions by lysyl endopeptidase proteolysis followed by SDS-PAGE. CPs were similarly obtained, but were not enzyme treated. As-1wk distinguished A, D, E and F from B and O, but not from each other. PcAs collected after several wk (As-swk) reacted with both HmCP and HmCCP. As-swk to A, D, E and F, cross-absorbed (xab) with heterologous CPs (HtCP), reacted with HmCPs and HtCPs not used for xab, but not with the HtCP used to xab. Apparently, As-1wk to each strain contained antibodies (Ab) only to N-terminus epitopes, whereas As-swk contained Ab to N-terminus and CCP epitopes. As-1wk and As-swk to A, D, E and F contained Abs specific to the N-terminus of the immunizing strain.

A226

EVIDENCE FOR VECTOR-SPECIFIC ACQUISITION OF BARLEY YELLOW DWARF VIRUS BY CEREAL APHIDS. F. E. Gildow, Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802.

Five species of cereal aphids, Rhopalosiphum padi, R. maidis, Sitobion avenae, Schizaphis graminum, and Metopolophium dirhodum, were fed seven days on California Red oats (Avena Byzantina L.) or Barsoy barley (Hordeum vulgaris L.) infected with the PAV, MAV, RPV, or RMV isolate of BYDV. Sixty aphids from each treatment were assayed for virus acquisition by immunoelectron microscopy and by hemolymph recovery bioassays utilizing microinjection. Of 20 aphid-virus combinations tested, only one (M. dirhodum-RPV) was negative for virus acquisition. When M. dirhodum were fed on purified RPV, virions failed to associate with the membrane of the hindgut and were not observed immunologically labelled in the hemocoel. Similar results were observed with brome mosaic virus, a nontransmitted virus. Results suggest that BYDV isolate recognition could occur at the hindgut. However, this recognition is not a determinate of transmission specificity.

A227

SUSCEPTIBILITY OF DARK GREEN AREAS TO SUPERINFECTION LEADS TO BREAKDOWN OF CROSS PROTECTION WITH STRAINS OF TOBACCO MOSAIC VIRUS (TMV). J.A.M. Rezende, and J. L. Sherwood. Oklahoma State University, Stillwater, OK 74078.

Cross protection between two serologically related strains of TMV (TMV-C and TMV-P) was studied in Nicotiana tabacum cvs. Samsun and Xanthi. Plants systemically infected with TMV-C or TMV-P and challenged over the entire leaf surface of 2 leaves with 1, 25, or 50 ug/ml of the other strain did not show protection 12-15 days after inoculation. When dark and light green areas were separately challenge inoculated with 1 ug/ml of virus, only plants challenged on dark green areas showed the presence of the challenge strain in the inoculated and upper leaves. A latent strain of TMV (PV-227) did not protect Samsun plants against TMV-C or TMV-P, when TMV-C or TMV-P replicated to detectable concentrations in the challenged leaves. The uneven distribution of the protective strain in the leaves may allow the establishment of the challenge strain, which leads to a breakdown of protection.

A228

MECHANICAL TRANSMISSION AND CHARACTERIZATION OF A CLOSTEROVIRUS FROM A GRAPEVINE LEAFROLL INFECTED GRAPEVINE. J. S. Hu, M. Wang, M. Maixner, and D. Gonsalves. Dept. of Plant Pathology, Cornell University, NYSAES, Geneva, NY 14456.

A closterovirus has been mechanically transmitted from a grapevine leafroll infected grapevine to *Nicotiana occidentalis*, where it induced necrotic local lesions on inoculated leaves; and curling, yellowing and mosaic symptoms on young leaves. The virus also induced very mild chlorosis symptom on young leaves of *N. banthamiana,* but was latent on *Vinca rosea, Datura stramonium, Gomphrena globosa, Cuburbita maxima,* and *Cucumis sativus.* The modal length of the virus is 800 nm, and in SDS-PAGE analysis, a 24 Kd coat protein band was identified. Several dsRNA bands ranging in molecular weight from about 3.5 x 10^6 to 5 x 10^6 Mr were isolated. Polyclonal antibodies were produced to the closterovirus in a rabbit, and used in double diffusion, ELISA, ISEM, and Western blot assays. The antibodies reacted with the closterovirus, and also with grapevine virus A and apple stem pitting virus. The relatedness of the virus with other similar closteroviruses, the distribution of the virus in grapevines, and the association of the virus with grapevine leafroll disease are under investigation.

A229

BIOLOGICAL FACTORS AFFECTING LEAFHOPPER TRANSMISSION OF PURIFIED MAIZE CHLOROTIC DWARF VIRUS (MCDV). R. Creamer[1], L. R. Nault[2], and R. E. Gingery[3]. USDA-ARS[3], Depts. of Plant Pathology[1] and Entomology[2], The Ohio State University, OARDC, Wooster, OH 44691.

Purified MCDV (WS strain), when acquired through membrane feeding, was transmitted by Graminella nigrifrons if the leafhoppers were allowed an initial acquisition on MCDV (M1 strain) -infected corn. Conversely, MCDV-WS-infected corn could aid transmission of purified MCDV-M1. After removal from MCDV-M1-infected plants, G. nigrifrons transmitted MCDV-M1 for up to 24 hours after feeding on healthy corn; the ability of the leafhoppers to acquire and transmit purified MCDV-WS was retained for 36 hours. In transmissions where G. nigrifrons fed initially on MCDV-infected plants and then on purified MCDV, purified MCDV could be transmitted without the helper virus, indicating that a factor from MCDV-infected plants other than virus assists in transmission of MCDV. Increasing the time for acquisition feeding of G. nigrifrons on MCDV-M1-infected corn or on purified MCDV-WS did not significantly change the transmission frequency of MCDV-WS. Amblysellus grex, an experimental vector of MCDV, also transmitted purified MCDV-WS after an initial acquisition feeding on MCDV-M1-infected corn.

A230

THE BREAKDOWN OF CROSS-PROTECTION AGAINST SEVERE FLORIDA STRAINS OF CTV. C. A. Powell, R. R. Pelosi, and M. Cohen. University of Florida, AREC, Ft. Pierce, Florida 34954

The ability of naturally occurring mild strains of citrus tristeza virus (CTV) to suppress the spread of severe strains of CTV into citrus propagated on susceptible rootstock was tested. Sour orange rootstock budded with Valencia sweet orange either infected with one of four mild strains of CTV or uninfected were planted at Ft. Pierce, FL where severe strains of CTV are prevalent. Decline was first observed 2.5 years after planting in both trees protected with mild isolates and in unprotected trees. After 6 years, the percentage of decline was 37.5, 39.8, 39.8, and 42.0 for the trees containing the 4 mild isolates of CTV, respectively, compared to 47.4 for the unprotected trees. After 8 years the percentages were 75.0, 75.7, 74.4, and 72.7, respectively, compared to 86.0 for the unprotected trees. These percentages were not significantly different. The breakdown of cross-protection was documented serologically using monoclonal antibodies which are specific for severe CTV strains occurring at Ft. Pierce, FL.

A231

PRELIMINARY IDENTIFICATION OF VIRUSES IN CERTAIN ORNAMENTALS SOLD IN CANADA. J.F. Peterson, Department of Plant Science, Macdonald College of McGill University, 21,111 Lakeshore Rd., Ste-Anne-de-Bellevue, Québec, Canada, H9X 1C0

Many of the cut flowers sold during the winter are imported. Striping of leaves and spathe shows on most irises, presumably Dutch bulbous iris, I. x hollandica, from The Netherlands. Results of host range tests and immunosorbent electron microscopy indicated that these irises were infected by a combination of iris mild mosaic (IMMV) and narcissus latent virus (NLV). A carlavirus, possibly lily symptomless virus, was revealed by electron microscopy of crude mince preparations of symptomatic leaves from European cut lilies, as well as some Asiatic hybrid lilies grown from imported bulbs. One cultivar of peony (Paeonia officinalis) imported for local multiplication showed severe peony ringspot symptoms, apparently associated with a strain of tobacco rattle virus (TRV). TRV has been recorded only once previously in Canada, and could pose an added threat to potato production.

A232

DETECTION AND HYBRIDIZATION ANALYSIS OF DOUBLE-STRANDED RNA ASSOCIATED WITH SOYBEAN DWARF VIRUS-INFECTED SOYBEAN. O. P. Smith[1], P. L. Hunst[2], A. D. Hewings[3], A. L. Stone[1], S. A. Tolin[2], and V. D. Damsteegt[1]. [1]USDA-ARS, Frederick, MD 21701, [2]VPI and SU, Blacksburg, VA 24061, and [3]USDA-ARS, Urbana, IL 61801.

Double-stranded (ds)RNA was isolated from leaves of 'Wayne' soybean infected with the dwarfing (D) or yellowing (Y) strain of soybean dwarf virus (SDV). Each strain produced two virus-specific dsRNAs. SDV-D dsRNAs were estimated to have molecular weights of 3.4 and 1.9 x 10^6 dal; corresponding dsRNAs for SDV-Y were 3.6 and 2.2 x 10^6 dal. Northern-blot hybridization analyses showed the strains share sequence homology and that the two dsRNAs correspond to viral genomic-length and 3' subgenomic-length species. Because SDV-D and SDV-Y are closely related serologically, immunological methods for in vitro strain differentiation are not practical. The ability to differentiate SDV-D from SDV-Y based on dsRNA profiles should facilitate investigations into the etiology of SDV-like viruses reported to occur in U.S. forage legumes.

A233

PARTIAL CHARACTERIZATION OF cDNA LIBRARIES TO AN ISOLATE OF ZUCCHINI YELLOW MOSAIC VIRUS (ZYMV) FROM FLORIDA. G.C. Wisler, E. Hiebert, and D.E. Purcifull. Department of Plant Pathology, University of Florida. Gainesville, FL 32611.

Complementary DNAs prepared to an aphid transmissible isolate of ZYMV from Florida were cloned in the expression vector λgt11. Clones representing the coding regions for the capsid protein (CP), cylindrical inclusion protein (CIP), and helper component (HC) were identified by serological detection of fusion proteins. Clones ranged in size from 0.6-4.5kbp and together represented ca. 80% of the genome. Sequences obtained showed 98% similarity of the CP with the 3' end of a Connecticut ZYMV isolate (Grumet & Fang, Phytopathology 79:1194.). A primer was developed from the ZYMV CIP sequence and was used to clone specifically for the 5'end of ZYMV using the λZapII vector. Clones from this study will be used to analyze the products encoded by the 5'end of the ZYMV genome.

A234

HEAVY METALS BIND TO RNA INSIDE MAIZE CHLOROTIC MOTTLE VIRUS PARTICLES AND REDUCE THE VIRION SURFACE CHARGE. R.N. Skopp, L.C. Lane, Dept. of Plant Pathology, Univ. of Nebraska, Lincoln, NE, 68583-0722.

Maize chlorotic mottle virus, a carmovirus, is more stable in acid than at pH 7. At pH 6 it has two anionic electrophoretic forms. Carboxypeptidase A converts the slow form to the fast form. Briefly heating virions at 50 C with heavy metals such as cobalt, zinc, or iron produces discrete forms with reduced mobilities. Heating metal ion treated virus with excess EDTA restores the "normal" electrophoretic mobility. Neither cysteines nor histidines appear to be involved in heavy metal binding. Heating MCMV in the presence of zinc ion hydrolyzes virion RNA. Model studies with hollyhock mosaic tymovirus show that zinc ion reduces the electrophoretic mobility of virion bottom component, but does not affect the mobility of top component. These studies suggest that heavy metal ions penetrate virion capsids, bind to RNA and reduce virion surface charge.

A235

ASSOCIATION OF POTATO VIRUS Y COAT PROTEIN WITH CHLOROPLASTS OF INFECTED TOBACCO. U.B. Gunasinghe and P.H. Berger. Plant Pathology/PSES, University of Idaho, Moscow, ID 83843

Chloroplasts from potato virus Y (PVY) infected, healthy or mock-inoculated tobacco (*Nicotiana tabacum* 'KY14') were isolated and treated with thermolysin to degrade coat protein (CP) adhering to their outer membranes. Using western blotting techniques, chloroplasts from infected tobacco yielded significant amounts of coat protein, whereas healthy and mock-inoculated controls had none. Using similar amounts of protein for SDS-PAGE, chloroplasts from infected plants had slightly less CP than total sap protein extracts. Reconstruction experiments and treatments with and without thermolysin confirmed that CP was specifically located within the chloroplast envelope. Additional experiments revealed the presence of helper component in chloroplasts. The function of these potyvirus gene products in chloroplasts is unknown; work is underway to create transgenic plants engineered to express these proteins in their chloroplasts.

A236

CELL-FREE TRANSLATION ANALYSIS OF THE BEET WESTERN YELLOWS VIRUS ST9 ISOLATE VIRION RNAs AND *IN VITRO* TRANSCRIPTS OF CAPSID PROTEIN ORF cDNA CLONES. L.-S. Chin, C. A. Blish, and B. W. Falk. Department of Plant Pathology, University of California, Davis, CA 95616.

Three cDNA clones representing the beet western yellows virus (BWYV) capsid protein ORF, readthrough region, and capsid protein ORF plus readthrough region were generated by Exo III deletion from a 3.2 kb 3' proximal clone and subcloned into pBluescript II sk+. *In vitro* transcripts were generated from these clones compared with the *in vitro* translation products from BWYV ST9 virion RNA. Three major protein products of ca. 66 Kd, 30 Kd, and 27 Kd were seen in the virion RNA preparations. A ca. 21 Kd product was generated from transcripts of the capsid protein ORF, and capsid protein ORF plus readthrough region. The ca. 21 Kd product specifically immunoprecipated with BWYV antiserum and demonstrates that the capsid protein was made only from transcripts and not from the virion RNA. Northern hybridization analysis using capsid protein cDNAs revealed the presence of a ca. 2.9 kb RNA in total RNA extracts from BWYV ST9-infected plants. These data suggest that the BWYV capsid protein is generated via a subgenomic mRNA strategy.

A237

POPULATION DYNAMICS OF VERTICILLIUM DAHLIAE AND ROOTS OF GOSSYPIUM HIRSUTUM. J.C. Broome, J.J. Marois, and K.G. Cassman. University of California, Davis, CA 95616

Verticillium dahliae microsclerotia and fertilizer were applied to soil in microplots at three 15 cm depth intervals. Nutrient placement affected host root length density, root diameter, and root infection. Fertilizer significantly increased the proportion of host root length density that occurred in the top (0-15 cm) and in the bottom (30-45 cm) interval. The percentage and total number of roots that were infected were reduced by the fertilizer treatment. Depth affected the pathogen propagule dynamics. The top interval (0-15 cm) contained the greatest in-season increase of microsclerotia, 5 to 13.2 ms/g. Using stepwise multiple regression, inoculum density and root radius explained 75% of variation in root infections. A log-log transformation of percent infection and inoculum density had a slope of 0.62, and explained 82% of the variation. A probability model explained 81% of the variation in root infections. Fertilizer reduced the propagule competency value and increased the rhizosphere width.

A238

SURVIVAL OF PYTHOPHTHORA CINNAMOMI IN RIVER AND FARM POND WATER USED FOR IRRIGATION IN SOUTH AFRICA. Sharon L. von Broembsen. Department of Plant Pathology, Stillwater, OK 74078-9947.

P. cinnamomi is prevalent in river systems of the southwestern Cape Province of South Africa, where river water is used for irrigating crops either directly or after holding in farm ponds. Natural populations of Phytophthora spp. declined to below detectable levels within five days after a pond was filled with highly infested (>1000 colony forming units/ L) river water and were reduced >98% after 7-10 days storage in the laboratory. Axenically produced P. cinnamomi zoospores and chlamydospores stored in filtered river water (FRW) survived longest at 8 and 12 C. For zoospores, P. cinnamomi could be recovered after 42 days in FRW at 8 C, but extinction was >98% after 10 days in all types of filtered irrigation water tested. Zoospores suspended in 5 um nylon mesh containers survived up to 11 days in a river and up to 17 days in a farm pond. Storage of infested river water in farm ponds for several weeks could greatly reduce inoculum in irrigation water taken from the surface of these ponds.

A239

CHARACTERIZATION OF GROWTH AND SPORULATION IN VITRO AMONG ISOLATES OF MYCOSPHAERELLA FIJIENSIS. Luis Jacome and Wolfgang Schuh, Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802.

Benomyl-sensitive and -resistant isolates of Mycosphaerella fijiensis were obtained from infected banana leaf tissue from Honduras, C. A. Fungal growth and conidia production on mycophyl agar were monitored over time at four temperatures (T) under 2.5 W/m^2 of continuous light. A computerized, video-image analysis system was used to assess growth. Concurrently, conidia were collected by brushing and washing of colonies. Conidia germinated from 20 to 35C with an optimum at 25C. Higher rates of growth were observed as T increased. No fungal growth was observed at 35C. Growth, expressed as the area under the fungal growth curve (AUFGC) responded linearly (R^2=0.8-0.98) at all T. However, AUFGC at 20C was better explained by a quadratic function (R^2>0.94). Cumulative spore production was linear against time (R^2=0.71-0.99) and AUFGC (R^2=0.78-0.99) at all T. Spore production decreased as T increased. Significant differences among isolates were observed.

A240

COFFE RUST (HEMILEIA VASTATRIX BERK. & BR.) EPIDEMIOLOGY IN COLOMBIA. J. Leguizamón, L. Orozco, L. Gómez and G. Cadena. Centro Nacional de Investigaciones de Café (Cenicafé). Chinchiná, Colombia.

Disease incidence, severity and latency period were studied at three locations with annual mean temperatures of 23°C, 21°C, and 19°C, associated with specific altitudes. Incidence and severity were highly correlated (r>0.8). Incidence and severity increased with increasing temperatures, while LP became shorter. Yield, foliage and disease incidence are closely associated. Therefore, leaf rust follows and intensifies the biennial crop pattern typical of coffee. The disease progress curve may differ among years and localities. However, in a typical crop season, the disease increases starting in June and reaches its highest values from November to January. During February, March and April, the disease incidence falls, mainly due to active foliage growth. In May and part of June disease incidence is at its lowest level. These results are being used for epidemic forecasting and for timing of fungicide sprays.

A241

CHARACTERIZATION OF TOMATO SPOTTED WILT VIRUS EPIDEMICS IN PEANUT. A. K. Culbreath[1], J. W. Demski[1] and J. W. Todd[2]. Department of Plant Pathology[1], and Department of Entomology[2], University of Georgia, Tifton, GA 31793-0748.

One field each (21.9 x 152.4 m) of Florunner and Southern Runner peanut (Arachis hypogaea L.) cultivars was divided into 240 contiguous plots (0.9 x 7.6 m). Spatial patterns and incidence of plants with spotted wilt symptoms were determined for each plot beginning 12 June, 1990 and at 2 wk intervals through 7 September. Diagnoses were confirmed using ELISA. Disease incidence increased throughout the season, but rates of disease progress were slower near harvest. Disease progress in Southern Runner was slower than in Florunner with final incidence averaging 1.5 and 3.1 symptomatic plants per plot respectively. Variance to mean ratios of final incidence were 2.1 and 3.6 for Southern Runner and Florunner respectively. Ordinary runs analysis and aerial photographs indicated clustered patterns of symptomatic plants in the field, suggesting the occurrence of secondary spread.

A242

ANALYSIS OF SPATIAL PATTERNS OF VEGETATIVE COMPATIBILITY GROUPS OF *CRYPHONECTRIA PARASITICA* USING MATRIX COMPARISONS. M. G. Milgroom, W. L. MacDonald and M. L. Double. Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853, and Division of Plant and Soil Sciences, West Virginia University, Morgantown, 26506.

Matrix comparison analysis was used to test whether vegetative compatibility groups (vcg's) of *Cryphonectria* (*Endothia*) *parasitica* occur in random patterns in natural stands of chestnut trees. Vcg's were determined for isolates from all cankers present in eight 20 x 20 m forest plots in West Virginia from 1977 to 1982. Vcg patterns were significantly nonrandom (i.e., aggregated) for at least one year during the study in 7 of 8 plots. Multiple occurrence of vcg's on the same tree was the major source of nonrandomness: all patterns were random when multiple occurrences were not included in the analysis. We hypothesize that conidia are an important source of inoculum on the same tree. However, there is no evidence for local nonrandom spread of asexual clones to nearby trees.

A243

ASSOCIATION OF COLLETOTRICHUM LAGENARIUM WITH ROOTS OF CUCURBIT CROPS AND WEEDS. C. L. Patterson, C. L. Sowell, and S. W. Hayes. Department of Plant Pathology, Oklahoma State University, W.W.A.R.E.C., P.O. Box 128, Lane, OK 74555.

Colletotrichum lagenarium (incitant of cucurbit anthracnose) was isolated from dark constricted lesions embedded with acervuli on roots of watermelon collected in southwestern OK. Cantaloupe, citron, and watermelon grown in potted soil contaminated with C. lagenarium-infected residue, conidia, or desiccated hyphae and acervuli developed symptoms identical to those observed in fields. Infection and symptoms did not develop on cucumber, honeydew melon, pumpkin, or squash. We concluded, therefore, that the primary inoculum of C. lagenarium was soilborne. During dry periods inhibitory to anthracnose epidemics inoculum likely was maintained in soil by root infection of some cucurbit crops and weeds.

A245

EFFECTS OF GENOTYPE FREQUENCY ON STRIPE RUST SEVERITY AND EFFECTS OF GENOTYPE FREQUENCY AND STRIPE RUST ON PLANT-PLANT INTERACTIONS IN WHEAT CULTIVAR MIXTURES. M. R. Finckh and C. C. Mundt. Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902.

Mixtures and pure stands of five wheat cultivars possessing different race-specific genes for resistance to Puccinia striiformis were grown in varying mixture ratios in the field at two locations. Yield components and disease severity were measured on individual plants on a per-cultivar basis. The effects of frequency and density of a genotype on disease severity were dependent on the genotypes in the mixtures and on the location, suggesting that factors other than frequency and diversity might affect disease severity. Plant-plant interactions between genotypes differed among different mixture ratios, between diseased and non-diseased mixtures, between years, and between locations. This suggests that biotic and abiotic environmental factors greatly affect plant-plant interactions in the field.

A246

EFFECTS OF VECTOR PREFERENCE FOR HEALTHY PLANTS ON PROGRESS OF STOCHASTIC EPIDEMICS. P. M. Burrows, K. S. Harrelson and O. W. Barnett.
Department of Experimental Statistics, Clemson University, Clemson, S. C. 29634-0367.

Acceleration or retardation of a stochastic epidemic, attributable to any factor, can be measured by ratios of mean times to epidemic completion for different levels of that factor. When disease is spread by a vector population, the effects of varying levels of vector preference for diseased and healthy plants, or of attractiveness and repulsion of vectors by diseased and healthy plants, measured in this manner, depend on population proportions of plants that are susceptible to the disease.

A247

EPIDEMIOLOGY OF PEANUT WEB BLOTCH IN EASTERN NEW MEXICO. C.M. Liddell, Department of Entomology, Plant Pathology and Weed Science, New Mexico State University, Las Cruces, NM 88003.

Web blotch is a serious foliar disease of Valencia peanuts in eastern New Mexico. The disease is currently controlled by scheduled fungicide applications. A prediction model is being designed to improve efficacy and reduce the frequency of fungicide applications. The causal agent in Phoma arachidicola and both teleomorph and anamorph stages are present in the field. The disease is favored by temperatures below 29C and diurnal cycles of relative humidity above 85% with periods over 95%. Conidia were detected on lesions from field plants at midseason and pseudothecia developed on necrotic tissue after incubation in the laboratory. In 1989, disease incidence was 100% in irrigated field plots and disease severity remained near 10-15% throughout the season. Overhead irrigation increased disease incidence to 100% within 5 days although disease severity remained low due to below normal rainfall.

A248

Use of stability analysis to predict the effects of soil moisture on yield of tobacco infected with Meloidogyne incognita. T. A. Wheeler[1], S. M. Schneider[2], K. R. Barker[1]. [1]Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616. [2]USDA-ARS, Oxford, NC.

Flue-cured tobacco (cv. NC27-NF) was transplanted into microplots infested with initial levels (Pi) of Meloidogyne incognita ranging from 0 to 20000 eggs/500 cm^3 soil. Numbers of nematodes/plant and leaf weight were determined by sampling destructively 10 times during 1988 and 1989. Stability analysis of 2-linked, differential equations representing the host-parasite system, showed that with moderate irrigation (0 to 41 kPa) a stable, nonoscillatory system was present. With low levels of irrigation (0 to 510 kPa) or Pi=20000, a stable, oscillatory system was common. With no water added, or plant-limiting nutrient deficiences present, unstable systems developed at Pi lower than 10000. Highest yields were associated with stable, nonoscillatory systems.

A249

A COMPARISON OF DISCRETE AND CONTINUOUS DISEASE SEVERITY KEYS FOR ACCURACY, PRECISION, AND PREDICTION OF YIELD LOSS CAUSED BY CORKY ROOT OF LETTUCE. R. Douglas O'Brien and Ariena H.C. van Bruggen, Department of Plant Pathology, University of California, Davis, CA 95616.

Six roots in each of three severity ranges (0-20, 20-80 and 80-100 % of taproot corked) were rated by 4 corky root experts, 3 plant pathologists and 3 novices using two discrete interval keys [a 7 level key developed for assessing corky root severity of mature lettuce plants (key1), and a 10 level key developed for screening germplasm for resistance 1 month after inoculation (key2)] and a 12 level Horsfall-Barratt continuous interval key based on % of the taproot area corked (key3). Roots 20-80% corked were scored less accurately and precisely than roots <20% or >80% corked. Key 3 was more accurate than the others at 20-80% corked. Novices were less accurate using keys 2 and 3 than others. In a 3 season field experiment, keys 2 and 3 predicted yield loss best at the rosette stage of lettuce growth (r^2=0.77 and 0.76, respectively). Key 1 was best at heading (r^2=0.51). At harvest, only keys 1 and 3 were useful (r^2=0.43 for both).

A250

ESTIMATION OF WHEAT LEAF RUST ON LEAVES AT DIFFERENT POSITIONS FROM WHOLE PLANT DISEASE DATA. K.V. Subba Rao, X.B. Yang, and J.P. Snow. Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

In modeling crop-pest interactions in cereals, disease data on different leaves on plants is needed. Crop models simulate plant growth by leaf development at different positions on plants. In conventional crop disease surveys, overall disease on a crop is recorded. A method to estimate the disease on leaves at different positions from the whole plant disease severity values and disease progress curves is needed. Leaf rust data collected during the 1986-87 and 1987-88 wheat growing seasons were used to study the relationship between rust on leaves at different positions and mean disease per plant. Theoretically, environmental effects are the same for different leaves at a given time, and therefore, the apparent infection rate (r) was assumed to be identical for all leaves. Development of leaf rust on the ith leaf was expressed as $dx_i/dt = r.n.X_t\ (1-x_{it})$ or in an integrated form $x_{it} = 1\text{- }Exp\ [-r.n.X_t(t - t_i)]$, where x_{it} is the rust on the ith leaf at time t, n is the number of leaves per plant, X_t is the mean disease on the plant at time t, and t_i is the time of leaf initiation. The equation fitted the field data and gave satisfactory prediction.

A251

COST/BENEFIT COMPARISON OF A WEATHER-BASED FUNGICIDE SCHEDULING PROGRAM VERSUS A CALENDAR SPRAY PROGRAM TO CONTROL LATE LEAFSPOT OF PEANUT. Forrest W. Nutter, Jr. and F. D. Mills. Dept. Plant Pathology, Univ. of Georgia, Athens, GA 30602 and Dept. of Agriculture, Abilene Christian University, Abilene, TX 79699.

Field experiments were conducted at Athens, Plains, and Tifton, GA, in 1988 and 1989 to compare a weather-based forecasting system with the currently used calendar-based fungicide spray program. In 1988 eight calendar based sprays were applied at each location whereas only three sprays were applied according to the forecast in Athens and Plains, and four sprays were used at Tifton. Seven calendar-based sprays were applied at each location in 1989; four sprays were applied using the forecasting model. Although disease levels at harvest were lowest when the calendar-based schedule was used, pod yields were equal to or slightly higher when the forecasting model was followed. Risk-rated yields were higher for the forecasting model (14 to 100 $ net return per ha higher than the calendar-based system), while the probability for profit remained unchanged (99% for both systems).

A252

CONVERSION OF CRYPHONECTRIA PARASITICA INDUCED CANKERS ON AMERICAN CHESTNUT STEMS. P. J. Bedker and R. L. Kovacs. Department of Plant Pathology, Rutgers University, New Brunswick, NJ 08903.

Vigorous American chestnut sprouts were inoculated on opposite sides of the stems at 46 and 76 cm from the ground with a virulent isolate of C. parasitica (SC58-19) from New Jersey. After 4 wks, the lower cankers were challenged with the isogenic, dsRNA containing hypovirulent isolate (NB88-58) on the margin perpendicular to the original inoculation. This challenge had a significant ($P < 0.01$) effect on canker expansion using regression analysis. Stems were removed over time, isolations were made from cankers, and conversion was determined by culture phenotype and spot hybridization analysis of ^{32}P-labeled recombinant plasmids to dsRNA. The first converted isolate from challenged cankers was obtained at day 6. At days 12 and 32 all isolates recovered were converted. In total for 13 harvest dates, 68 of 147 (46.3%) and 2 of 168 (1.2%) of the isolates from the lower and upper cankers were converted, respectively.

A253

OCCURRENCE OF BASAL DECAY IN WESTERN HEMLOCK AS A FUNCTION OF TREE HEIGHT AND DIAMETER. D.C. Shaw and R.L. Edmonds, College of Forest Resources, AR-10, University of Washington, Seattle, WA, 98195.

Heterobasidion annosum and Armillaria species cause basal decay in coastal western hemlock. We randomly sampled 35 year-old trees in precommercially thinned and unthinned forest stands 20 years after thinning on the Olympic Penninsula, Washington. Armillaria sp. was consistently present only on short small diameter trees or trees with other decay infections. H. annosum was present on trees covering the range of heights and diameters. This supports the present understanding of Armillaria as an opportunist and attacker of lower vigor trees in western Washington. H. annosum, however, can successfully attack trees of any height and diameter. Analysis of the distribution of basal decays by height and diameter of host trees aids in understanding the ecology of these decay organisms.

A254

EVIDENCE FOR HERITABLE VARIATION IN RESISTANCE TO BLIGHT IN CHINESE CHESTNUT. Frederick V. Hebard. American Chestnut Foundation, Rte 1, Box 17, Meadowview, VA 24361.

Arthur Graves produced a number of first hybrids of Chinese and American chestnut between 1937 and 1939. Twenty-five of these were planted at the White Memorial Foundation in Litchfield, CT, in the late 1940s. Sixteen were surviving in 1989. Four of the surviving trees are progeny of one Chinese chestnut parent, tree R1T4, and five are progeny of a second parent, tree R1T7. The American chestnut male parent may have differed depending on the Chinese chestnut female parent. Four of five of the original stems with Chinese parent R1T7 were living in 1989, whereas all four of the original stems with Chinese parent R1T4 were killed by naturally occurring chestnut blight; the trees survive due to sprouts which originated after the original stem was killed. These data suggest that there are heritable differences in blight resistance among Chinese chestnut trees.

A255

NEW ASSOCIATIONS OF PITCH CANKER CAUSED BY FUSARIUM SUBGLUTINANS WITH BARK, TWIG, AND CONE BEETLES. J. W. Fox, M. E. Schultz, T. R. Gordon and D. L. Wood, Department of Entomology and Plant Pathology, University of California, Berkeley.

Pitch canker of Monterey pine recently appeared in Santa Cruz County, where it is associated with the feeding activity of scolytid and anobiid beetles. Fusarium subglutinans was isolated from 15-25% of both twig and engraver beetles (Pityophthorus carmeli, Ips mexicanus, I. paraconfusus and Ernobius punctulatus). The fungus was isolated from less than 10% of the cone beetle, Conophthorus radiatae. It was isolated from the twigs, branches and cones these beetles infested. Cone whorls accounted for 30-60% of the infection courts, depending on the site. We hypothesize that cone and twig beetles spread the fungus, while Ips spp. kill diseased trees. Monterey pine has continuous cone production, possibly because of its short life span relative to other pines.

A256

WATER CONTENT DETERMINATIONS OF SAND BY MRI. J. S. MacFall, G.A. Johnson, P.J. Kramer. Duke University, Durham, NC 27706

Regions of water depletion surrounding roots of loblolly pine seedlings were observed with magnetic resonance imaging (MRI). The relationship between signal intensity, water binding, water content, and instrument settings is complex. However, a linear relationship was found between water content of wet sand (5-25% water) and signal intensity. This presents the possibility of determining water content from signal intensity normalized against a reference material. A 5mm diameter NMR sample tube filled with a reference solution was placed in the field of view of the wet sand. For a pulse sequence repetition time of 800-3200 ms at 2T, a linear relationship was observed between water content and signal intensity normalized against a 25% CuSO4 (.007M)/75% D2O solution. A curvilinear relationship fitting a polynomial function was found for water content and signal intensity normalized against a solution of 25% CuSO4 (.003M)/75% D2O. These relationships are being studied further, with the objective of quantitatively describing water uptake dynamics of plant roots.

A257

SPECIFIC DIAGNOSIS OF ASH YELLOWS BY MEANS OF BIOTINYLATED CLONED DNA PROBES. **R. E. Davis, W. A. Sinclair[1], I-. M. Lee, and E. L. Dally. Microbiology and Plant Pathology Laboratory, Agricultural Research Service, USDA, Beltsville, MD 20705, and [1]Department of Plant Pathology, Cornell University, Ithaca, NY 14853.**

Cloned biotin-labeled fragments of DNA from Catharanthus roseus infected with an AshY MLO were employed as probes in dot hybridizations. Four probes all hybridized with nucleic acid from C. roseus containing the AshY MLO but not with nucleic acid from healthy C. roseus. These probes were shown to contain chromosomal DNA of the MLO. Under stringent conditions of hybridization (52 C), none of the probes hybridized with nucleic acid from C. roseus carrying the MLOs associated with 10 other plant diseases. Two probes were tested for diagnosis of AshY in naturally infected white ash (Fraxinus americana). These probes hybridized with nucleic acid from diseased trees but not with that from healthy ash. These probes will facilitate searches for insect vector(s) and alternative plant hosts of AshY MLOs.

A258

TRICHOSPORIUM SYMBIOTICUM AND OTHER FUNGI ASSOCIATED WITH THE FIR ENGRAVER BARK BEETLE, SCOLYTUS VENTRALIS. William J. Otrosina and George T. Ferrell, Research Plant Pathologist and Research Entomologist, USDA Forest Service, 1960 Addison Street, Berkeley, California, 94701.

Isolates of T. symbioticum (Ts) and other fungi were obtained from beetles emerging from a provenance study plantation of white fir trees undergoing attack by the fir engraver. Additional isolates of Ts were obtained from infested stands in selected geographic areas corresponding to seed sources of the provenance plantation. Growth rate of Ts on 1.25% malt extract medium amended with 800 ug/ml cycloheximide was about 80% of non-amended medium. Cultures of Ts isolates from different geographic areas differed in rates of pigment development and growth on both media. Also, morphological features of conidiophores and conidia of this fungus do not conform to descriptions of other Trichosporium species. In the provenance study plantation, the insect parasite Beauvaria bassiana was isolated in high frequency from emergent beetles and may relate to population density of the bark beetles.

A260

OUTPLANTING SURVIVAL OF BLACK SPRUCE SEEDLINGS LIFTED FROM NURSERY FIELDS WITH CYLINDROCLADIUM ROOT ROT. J. E. Saunders[1], J. Juzwik[2], and R. Hutchison[3], [1]Forest Health and Protection Section and [3]Ontario Forest Research Institute, Min. of Nat. Res., Sault Ste. Marie, Ontario, Canada P6A 5N5, and [2]USDA For. Serv., North Cen. For. Expt. Sta., St. Paul, MN 55108.

Black spruce seedlings lifted from compartments known to have Cylindrocladium root rot at two Ontario forest nurseries were outplanted and monitored for survival and growth through two growing seasons. Significantly higher levels of mortality caused by Cylindrocladium floridanum Sob. & C.P. Seym. ($p<0.05$) occurred in seedlings with symptomatic shoots or with main root lesions and non-symptomatic shoots, compared to seedlings with only lateral root lesions and non-symptomatic shoots or non-symptomatic roots and shoots. More than 30% of the outplanted seedlings died by the end of the second growing season; > 76% of this mortality occurred in the first 6 months. Differences in annual growth increment among the four categories of surviving seedlings were not significant at the end of the second season.

A261

TOMATO MOSAIC TOBAMOVIRUS TRANSMITTED FROM WATERS IN THE ADIRONDACK MTS. V. Jacobi and J.D. Castello. SUNY College of Environmental Science and Forestry, Syracuse, NY 13210.

Tobamoviruses, consistently recovered from waters draining forest stands in the Adirondack high peaks, were identified as tomato mosaic virus (ToMV) based upon serological and host range tests. Three water isolates were compared with the L-strain, and dogwood and lilac isolates of ToMV by host range, agar gel double diffusion (AGDD) and indirect ELISA. In reciprocal AGDD, tests all six isolates were identical. In indirect ELISA, however, the L-strain and the lilac isolate were identical to only one of the water isolates and could be distinguished from the other two isolates by differential reciprocal dilution endpoints against twelve tobamovirus antisera. The three water isolates also were distinguishable from the lilac isolate by symptom production in indicator plants. ToMV was consistently detected by ELISA in the roots but not the needles of inoculated balsam fir (*Abies balsamea* (L.) Mill.) seedlings five months post inoculation.

A262

TOMATO MOSAIC TOBAMOVIRUS TRANSMITTED FROM LILAC. J.D. Castello and C.R. Hibben. SUNY College of Environmental Science and Forestry, Syracuse, NY 13210 and Brooklyn Botanic Garden Research Center, 712 Kitchawan Rd., Ossining, NY 10562

A virus was mechanically transmitted to Xanthi tobacco from symptomatic foliage of lilac, *Syringa* x *nanceiana* McKelvey cv 'Rutilant' growing at the Arnold Arboretum, Jamaica Plain, MA. Field symptoms consisted of foliar chlorotic mosaic and mottle. Dieback subsequently developed. Because the shrub also was infected with MLOs, as determined by DAPI, it was not possible to attribute particular symptoms to virus infection alone. Based upon host range, symptomatology, and serological tests the virus was identified as tomato mosaic virus. The virus was back transmitted to healthy seedlings of *S.* x *henryi* cv 'Lutece' and white ash (*Fraxinus americana* L.). No symptoms developed in lilac but a systemic chlorotic mottle and mosaic developed in white ash. The virus was subsequently recovered from young foliage of both lilac and white ash.

A263

DNA RESTRICTION FRAGMENT ANALYSIS OF *NECTRIA COCCINEA* VAR. *FAGINATA* AND *N. GALLIGENA* ASSOCIATED WITH BEECH BARK DISEASE. E.M. Mahoney, USDA Forest Service, Hamden, CT 06514, and Cornell University, Department of Plant Pathology, Ithaca, NY 14853.

Both *Nectria coccinea* var. *faginata* (Ncf) and *N. galligena* (Ng) kill American beech (*Fagus grandifolia*) infested by the beech scale (*Cryptococcus fagisuga*) in North America. Ng is a globally distributed pathogen on many arboreal species, but Ncf is known only on American beech and only in northeastern North America. The phylogenetic relationship of these fungi was explored through restriction fragment analysis of total genomic DNA from single-ascospore isolates obtained from American beech. Southern blots representing four different restriction enzymes were probed with randomly selected low-copy number, nuclear DNA clones derived from isolate EM-252 of Ncf. Analyses indicated that Ng is the more variable species and that Ncf has not evolved recently from Ng.

A264

RESPONSE OF FOUR TREE SPECIES TO VARYING DOSES OF OZONE IN NORTHCENTRAL PENNSYLVANIA IN 1988 AND 1989. M. Simini, J. M. Skelly, and D. D. Davis, Department of Plant Pathology, 211 Buckhout Lab, The Pennsylvania State University, University Park, PA 16802.

Four 2-year-old seedlings each of Prunus serotina Ehrh., Liriodendron tulipifera L., Quercus rubra L. Wangenth., and Acer rubrum L. were planted in each of 16 plots in a randomized complete block design at 3 sites (lat. 41° 19', long. 79° 02'; lat. 41° 07', long. 78° 31'; lat. 41° 20', long. 77° 26') in northcentral PA. Seedlings were exposed to ambient air or to charcoal filtered air in open-top field exposure chambers containing 95%, 60% or 40% of the ozone (0_3) in ambient air. The total 0_3 dose was greater at the western sites than at the eastern-most site and 0_3 was much greater in 1988 than in 1989. Foliar stipple injury of P. serotina and L. tulipifera was correlated positively with 0_3 dose at all sites in 1988 and 1989, and premature coloration and abscission of foliage was correlated positively with 0_3 dose at the high 0_3 site during both growing seasons. Seedlings of Q. rubra and A. rubrum were unaffected by elevated levels of 0_3 in this study.

A265

Relationships among forest litter burning, seedling density, and black cherry leafspot severity in northern Pennsylvania. G. Stanosz, PA Bur. For., 34 Airport Dr., Middletown, PA 17057.

Leafspot, caused by Blumeriella jaapii (Rehm.) v. Arx, causes defoliation and death of black cherry (Prunus serotina Ehrh.) seedlings. Litter in ten 5-m radius plots in a maturing stand was burned prior to seedling emergence; ten similar plots were not burned. Survival, number of leaves, and disease severity (foliage estimated visually as $\leq$10%, 11-50%, or $\geq$51% symptomatic) were recorded on four dates for the same 25 current-year seedlings/plot. Black cherry seedlings were counted in midsummer in the square foot surrounding each sample seedling. Numbers of living sample seedlings, sample seedlings with leaves, leaves/sample seedling, and sample seedlings in the lowest severity class, were greater in burned than unburned plots on all dates. These variables tended to be correlated positively with seedling density in burned plots (mean=2.5) and negatively with seedling density in unburned plots (mean=8.1). Practices that reduce pathogen survival (in leaf litter) and seedling density may lessen leafspot's impact on regeneration.

A267

RUST (PUCCINIA GRINDELIAE) OF BROOM SNAKEWEED, A RANGELAND WEED IN NEW MEXICO. J.P. McEntee and C.M. Liddell, Department of Entomology, Plant Pathology and Weed Science, New Mexico State University, Las Cruces, NM 88003.

A potential biocontrol agent, Puccinia grindeliae, is being evaluated on Broom Snakeweed (Gutierrezia sarothrae), a competitive shrub which over the past 100 years has displaced forage grasses on New Mexico rangeland. The rust is native to New Mexico and occurs at low levels in many parts of the state. Surveys of Broom Snakeweed populations infected with rust indicate that this disease occurs in small clumps with a highly infected host as the clump focus. Infected plant material in the field bears teliospores and spermatia, but not uredospores, which are lacking in P. grindeliae. Leaves, flowers and stems can be infected. Basidiospores have been observed to develop rapidly from teliospores incubated under fluorescent lights at 25C and 90 and 100% relative humidity.

A269

GENETIC CONTAINMENT SYSTEMS FOR BIOCONTROL AGENTS. D. C. Sands and R. V. Miller. Dept. of Plant Pathology, Montana State University, Bozeman 59717, and Mycogen Corporation, Ruston, LA 71270.

Biocontrol agents, whether natural or genetically manipulated, cannot be released until they are considered environmentally safe. Yet, it is impossible to adequately assess risks with our present technology due to limited conclusions possible with microcosm studies and the inability to detect minute numbers of a given microbe. Combined with the lack of prior data bases, it is difficult, if not impossible, to predict the impact of releasing most biocontrol agents. We propose that microbes be genetically constrained, at least during preliminary small-scale releases aimed at ascertaining safety and efficacy. Such constrained microbes could include auxotrophic mutants requiring substances that are not commonly free in the environment, temperature-sensitive isolates, non-sporulating strains, isolates incapable of surviving drying, conditional lethal mutants, and/or use of suicide plasmid systems.

A270

CONIDIATION OF COLLETOTRICHUM TRUNCATUM IN SUBMERGED CULTURE: THE NUTRITIONAL ENVIRONMENT INFLUENCES CONIDIAL GERMINATION AND DISEASE DEVELOPMENT ON SESBANIA EXALTATA. D.A. Schisler, M.A. Jackson, and R.J. Bothast, USDA-ARS, NRRC, Peoria, IL 61604.

Colletotrichum truncatum has considerable potential for development as a mycoherbicide for controlling the noxious weed, hemp sesbania (Sesbania exaltata). Conidia of C. truncatum (NRRL 13737) were produced in semi-defined liquid media with C:N ratios of "40:1", "15:1", and "5:1". Conidia produced in "5:1" medium were longer and thinner than "15:1" and "40:1" conidia and a higher proportion contained 2, rather than 1, nuclei per conidium. After either 6 or 12 h on cellulose membranes, a greater proportion of "5:1" conidia germinated than "15:1" and "40:1" conidia, but treatments did not differ when germination was assayed on attached S. exaltata leaves. Results from equality of variance tests implied that the leaf environment had the greatest influence on "15:1" conidial germination. All conidial treatments reduced plant growth, though "5:1" and "15:1" conidia induced greater reductions in shoot height and shoot dry weight than did "40:1" conidia.

A271

SUBMERGED-CULTURE SPORULATION IN ALTERNARIA SPP. AND IDENTIFICATION OF NEW CONIDIA BY FLUORESCENCE MICROSCOPY. K.M. Howard, M.G. Smart, and R.J. Bothast, USDA-ARS, NRRC, Peoria, IL 61604.

Alternaria crassa and Alternaria cassiae are important fungal pathogens of Jimsonweed (Datura stromonium) and Sicklepod (Cassia obtusifolia), respectively. Both show potential for use as mycoherbicides. Failure to produce conidia in submerged culture has limited commercialization of these weed pathogens. Studies were conducted in column bioreactors containing minimal medium (MM) or Richards V-8 (RV-8). Conidial inocula were stained with Nile red fluorescent dye and added to the column bioreactors yielding final concentrations of 1.2×10^4 and 8.3×10^3 conidia/ml for A. crassa and A. cassiae, respectively. Between $1.5 - 3.5 \times 10^3$ conidia/ml were recovered for each fungus after 96 h in MM and approximately 30% of the total spores recovered were identified as new

conidia. New conidia were distinguished from inocula by their inability to fluoresce. This is the first report of either fungus sporulating in liquid culture.

A272

DEVELOPMENT OF EMERGENCE-PROMOTING RHIZOBACTERIA FOR SUPERSWEET CORN. E.M. Tipping, D.C. Covert, E.E. Onofriechuk, R.M. Zablotowicz and J.W. Kloepper.

Supersweet corn, especially hybrids containing the SU-2 gene, is prone to stand establishment problems. We initiated a program to select emergence-promoting rhizobacteria to improve establishment. Greenhouse trials indicated that several Serratia and Pseudomonas strains were able to elicit significant effects on emergence, final stand and vegetative biomass, although some variation in performance could be attributed to cultivar. In initial field trials in Florida (1988), several strains, including S. proteamaculans strain 1-102 and P. putida strains GR12-2 and 61-9A elicited significant effects on emergence. Ontario field trials in 1988 demonstrated a significant increase in final stand by P. fluorescens 31-12 and S. liquifaciens 2-68. Both strains as well as 1-102 also had a significant effect on yield. Subsequent studies were focused on compatibility with the fungicide Captan, and in 1989 Ontario field trials, strain 1-102 elicited a significant increase in stand relative to the Captan control when applied to treated seed.

A273

DUAL-STAIN FLUORESCENCE MICROSCOPY TO OBSERVE THE INTERACTION OF PYRENOPHORA WITH A BIOCONTROL FUNGUS IN WHEAT STRAW. W.F. Pfender, J. Rabe, and L. King. Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502.

Pyrenophora tritici-repentis, causal agent of wheat tan spot, was visualized by means of indirect fluorescent antibody staining, using antibodies produced in chicken and anti-chicken IgG conjugated to fluorescein as FITC. Limonomyces, a basidiomycete that can reduce ascocarp production by Pyrenophora in straw, was stained with rhodamine (as TRITC) conjugated to wheat germ agglutinin. The interaction was observed in sterilized wheat straw inoculated with the two fungi. To prevent autofluorescence of plant tissue from masking the TRITC staining, the tissue was counterstained with toluidine blue-O. With this method, we observed that the fungi have different colonization patterns. Also, Limonomyces commonly grew in close association with Pyrenophora, and could occasionally be seen growing inside the hyphae of the pathogen as early as 24 hr after their mycelia had grown into close proximity.

A274

Development of media to produce conidial biomass of Trichoderma harzianum for biological control. G.E. Harman, X. Jin, T.E. Stasz, G.P. Peruzzotti and A.G. Taylor. Cornell University, Geneva, N.Y. 14456 and Eastman Kodak Co., Rochester, NY 14650.

Biomass of organisms to be used in biocontrol must be inexpensive to produce in liquid fermentation, capable of withstanding drying, insensitive to environmental fluctuations, and have a long shelf life. Different minimal media were compared and minimal media such as Czapek Dox or Richard's media were found to produce high proportions of conidia, but overall yields were low. Addition of V8 juice to these media increased yields by 8 to 16-fold, but only 1 to 10% of the conidia were viable after vacuum drying. However, addition of osmoticants, i.e. polythylene glycol (PEG), $MgCl_2$, or mannitol to Richard's medium with V8 juice (RM8), provided a high level of conidial production, and these conidia were resistant to vacuum drying. Further, conidia produced in RM8+PEG were insensitive to storage relative humidity, more effective in biocontrol seed treatment of cucumber against infection of Pythium than those produced in RM8, and survived longer on treated seeds.

A275

BIOLOGICAL AND CHEMICAL CONTROL OF RHIZOCTONIA SOLANI AG-4 IN SNAP BEAN. D. R. Sumner[1], J. A. Lewis[2], and R. D. Gitaitis[1]. [1]Department of Plant Pathology, University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793 and [2]USDA/ARS, Beltsville, MD 20705.

Snap bean cv. Strike was grown in field plots of Tifton loamy sand infested with Rhizoctonia solani anastomosis group (AG)4, as a double-crop following corn for three consecutive years. Flutolanil, PCNB, metalaxyl, metalaxyl + flutolanil, Gliocladium virens (G1-21), Trichoderma hamatum (TRI-4), the binucleate Rhizoctonia-like fungus CAG-2, and Pseudomonas cepacia were applied as treatments for two or more years. Flutolanil consistently increased plant stand, reduced populations of R. solani in soil and increased yield of green pods compared with the control. Flutolanil was significantly more effective than PCNB in 2 of 3 yrs, but yields were not different. Biologicals were variable, but each treatment increased plant numbers in 1 of 3 yrs, and G1-21, CAG-2, and P. cepacia increased yield in 1 of 3 yrs.

A276

DAMPING-OFF OF MARIGOLD IN RELATION TO MICROBIAL RECOLONIZATION OF STEAMED AND FUMIGATED SOILS. T. Isakeit, A. R. Weinhold, J. G. Hancock, and M. N. Schroth, Department of Plant Pathology, University of California, Berkeley, CA 94720

The incidence of damping-off of marigold following the addition of Rhizoctonia solani to two soils which had been steamed or fumigated with methyl bromide/chloropicrin was compared with the microbial recolonization of these soils. Disease incidence was highest in freshly steamed and fumigated soils, which coincided with a marked reduction in bacterial, fungal and actinomycete populations. The addition of 1% non-treated soil (w/w) to freshly steamed or fumigated soil resulted in an increase in the population of bacteria and a decrease in disease incidence. The population of fungi or actinomycetes did not increase. A steamed soil which was recolonized nine weeks by survivors was more suppressive to damping-off than the non-treated soil. In this soil, microbial populations were at the same level as non-treated soil, but species diversity was lower. These results suggest that microflora can be manipulated in treated soils to make them suppressive to soilborne pathogens.

A277

BIOLOGICAL CONTROL OF ROOT-ROT AND PRE-EMERGENCE DAMPING-OFF OF WHITE BEAN WITH PLANT GROWTH-PROMOTING RHIZOBACTERIA. M.S. Reddy, S. Young and G. Brown, Microbial Inoculants, Allelix Crop Technologies, 6850 Goreway Dr., Mississauga, Ontario, L4V 1P1.

Alternative methods for control of the soil-borne plant pathogens Fusarium solani, Rhizoctonia solani and Pythium ultimum are being sought since adequate chemical control has not been achieved, and there is heightened concern over toxic accumulations of agri-chemicals. More than 100 plant growth-promoting rhizobacterial strains (PGPR) were screened for their ability to protect white bean seedling roots from these three pathogens. Results indicate that mode of application has a major impact on the efficacy of PGPR strains. There is a positive correlation between the ability of strains to inhibit fungal growth on solid media and their ability to suppress pathogen activity on white bean seedlings. Similarly, there is a correlation between antibiosis in liquid broth and the application of PGPR to pathogen-infested soil. In most cases, strains which were particularly effective as soil treatments were not as effective when applied as a seed treatment.

A278

STUDIES ON THE MECHANISM OF HIGH VARIATION IN *PYRICULARIA ORYZAE* (*MAGNAPORTHE GRISEA*). B. C. Wu and C. W. Magill, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843

Many mechanisms (i.e. mutation, heterokaryosis, aneuploidy, sexual and asexual recombination) have been proposed, but none fully explains, the unusually high pathotype variation observed in *Pyricularia. oryzae*. Careful examination of monoconidial cultures of unstable isolates over 4 generations revealed an apparent mutation rate of one out of sixty. Variability is not random in the population, but is exhibited only in progeny from some individuals. Frequent reversal to the parental type is observed, and chromosome rearrangements are associated with pathogenicity changes. We propose that a transposable element is present in the genome of *P. oryzae* and that it is active in unstable strains. Insertion into an avirulence allele can inactivate it, causing a change in pathotype. To test this hypothesis, we are examining both stable and unstable isolates from the same monoconidial culture for genomic differences at the molecular level.

A279

MOLECULAR POLYMORPHISMS ASSOCIATED WITH SECTORING OF *COLLETOTRICHUM GLOEOSPORIOIDES* CAUSING POST BLOOM FRUIT DROP OF CITRUS. H.D. Liyanage, R.T. McMillan, R.M. Sonoda and H.C. Kistler. Dept. of Plant Pathology, University of Florida, Gainesville, FL 32611.

Colletotrichum gloeosporioides isolates causing post bloom fruit drop of citrus show variability in colony characteristics (sectoring) and virulence. Isolates from diseased plants from Florida and different geographic locations of the world have been tested for polymorphism in 26S ribosomal DNA (rDNA), p-nitrophenyl butyrate esterase (PBE) activity and chromosome-

sized DNA molecules separated by CHEF gel–electrophoresis. Preliminary observations based on rDNA restriction fragment length polymorphism using a *Neurospora crassa* 26S rDNA probe suggest that two distinct forms of rDNA are present in *C. gloeosporioides*. DNA derived from single–spore isolations of sectored cultures may contain only one of the two forms of rDNA, and isolates may show greater than 50% reduction in PBE activity compared to wild type. Experiments in progress seek to understand the vegetative rearrangement of rDNA and PBE activity by examining the molecular karyotype of wild type and derived strains.

A280

ANALYSIS OF CHROMOSOME-SIZED DNA MOLECULES IN STRAINS OF TWO FORMAE SPECIALES OF FUSARIUM OXYSPORUM AND PROTOPLAST FUSION PRODUCTS E.A. Momol, F.N. Martin, J.W. Kimbrough, and H.C. Kistler, Department of Plant Pathology, University of Florida, Gainesville, FL 32611.

To determine if genetic recombination could occur, protoplasts of Fusarium oxysporum f.sp. conglutinans strain ATCC 9990 (a cabbage pathogen), and of F. oxysporum f.sp. raphani strain ATCC 16601 (a radish pathogen) were fused in the presence of PEG. Fusion was confirmed by restriction fragment length polymorphism (RFLP) analysis of nuclear DNA. Chromosomes of parental strains and single-spored derived cultures from fused strains were separated using a contour clamped homogenous electrical field (CHEF) gel electrophoresis. Chromosome-sized molecules differed greatly in mobility between parental strains, with a minimum of 11 and 8 bands detected for ATCC 16601 and ATCC 9990, respectively. Chromosomal banding patterns of fusion products were identical to parental strains.

A281

RFLP GROUPS AND PHYSICAL MAP OF MITOCHONDRIAL DNA FROM *FUSARIUM OXYSPORUM* F. SP. *NIVEUM*. D. H. Kim, R. D. Martyn, and C. W. Magill, Department of Plant Pathology and Microbiology, Texas A&M University, College Station 77843.

A restriction map of mitochondrial DNA (mtDNA) from *Fusarium oxysporum* f. sp. *niveum*, causal agent of Fusarium wilt of watermelon, was constructed to facilitate understanding of the relationship among races and to identify variable regions in mtDNA among RFLP groups. Mitochondrial DNA was isolated from a race 0 isolate and digested with four restriction enzymes: *Eco*RI, *Hin*dIII, *Hpa*I, and *Pst*I, either singly or in selected pairs. The digestions yielded 4, 9, 21, and 10 fragments with the respective enzymes. Restriction patterns of mtDNA were examined on agarose gels and by hybridization with cloned *Pst*I fragments [pFON1 (1.0kb) - pFON9 (9.1kb)]. The hybridization pattern with each *Pst*I fragment probe was used to determine the physical order of restriction sites based on overlapping regions. Among the cloned *Pst*I fragments, fragment pFON3 (1.5kb) had homology with itself and the 9.1kb and 2.0kb fragments and seems to be an important region in discriminating among the five RFLP groups detected within the 44 isolates examined. The RFLPs among the isolates appeared to be primarily a result of base substitutions within the mtDNA; however, one insertion and one deletion event was detected in two of the isolates.

A282

ISOLATION AND CLONING OF A PROTEIN KINASE HOMOLOG FROM COLLETOTRICHUM TRIFOLII. N. L. Brooker and M. B. Dickman. University of Nebraska, Dept. of Plant Pathology, Lincoln, NE 68583-0722.

Protein kinases mediate extracellular and intracellular transduction signals in eukaryotic cells. We are searching for fungal genes encoding such kinases and intend to examine their roles in environmental, developmental, and pathogenic signal pathways. This study uses the race-cultivar specificity of Colletotrichum trifolii and its host alfalfa. Genomic libraries of both fungal races have been constructed. Both race 1 and 2 libraries were then sequentially screened using modified homology probing techniques, with a pair of degenerate oligonucleotide probes. These probes corresponded to separate, distinct highly conserved sequences within the central core of the catalytic domain of mammalian protein serine kinases. A 5 kb DNA fragment was subcloned from a single race 1 plaque which hybridized to both probes. DNA sequence analysis showed that this cloned region encodes a number of conserved protein kinase motifs. The clone will be used to elucidate the biochemical function of this putative protein kinase.

A283

ISOLATION OF PUTATIVE PROTEIN KINASES BY THE POLYMERASE CHAIN REACTION. M. B. Dickman, A. R. Schutz, and C. J. Stryker, Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722.

Protein kinases are pivotal in cellular regulatory processes including the transduction of external environmental signals. Since involvement of these enzymes in the general signal transduction pathway has been shown to occur in an extremely wide array of organisms, it is likely that in fungal phytopathogens protein kinases are similar in action. Functional studies of protein kinases could be greatly facilitated by the molecular cloning of the genes encoding these enzymes. Using the Colletotrichum trifolii-alfalfa race specific system, strategies have been initiated to examine the role of the protein kinase C family as part of the signalling pathway. Fully degenerate oligonucleotides corresponding to two conserved amino acid motifs of protein kinase served as primers for the polymerase chain reaction. Sequences analysis has revealed characteristic domains of protein kinase C suggesting that the fungal homologues may likewise function in the transduction of external signals.

A284

TARGETING SPORULATION SPECIFIC GENES FOR DISEASE CONTROL. R. A. Dean[1], M. Stringer[2], T. Sewell[2] and W. E. Timberlake[2]. [1]Dept. of Plant Pathology, Clemson Univ., SC . [2]Depts. Plant Pathology and Genetics, Univ. of Georgia, GA .

Increasing concern about food safety necessitates the need for new, safe disease control strategies. One approach is to target genes controlling fungal propagation and dissemination. Several important genes regulating sporulation have been isolated from Aspergillus nidulans and have been used to identify corresponding genes from closely related pathogenic fungi. Other sporulation specific genes have been cloned, but nothing is known about their function. One such gene, CAN41, is expressed to high levels early during conidiogenesis in A. nidulans. Sequence analysis reveals two open reading frames. The first potentially encodes a hydrophobic protein of 15.6 kDa., pI 4.5 and the other encodes a highly basic protein of 11.1 kDa, pI 12.1. Both reading frames contain the same two small introns, with splice junctions typical for A. nidulans. Forced expression of CAN41 in vegetative hyphae results in reduced hyphal extension, but does not initiate asexual reproduction. When CAN41 is deleted, conidiophores appear darker and encased in a droplet of liquid. The conidia themselves are less hydrophobic than in the wild type. Electron micrographs show the conidial cell walls lack the hydrophobic rodlet layer. We propose that CAN41 is required for a structural component of the conidial cell wall and may contribute to the survival of the conidia.

A285

COMPARISON OF PROMOTER STRENGTH IN *COCHLIOBOLUS HETEROSTROPHUS*. Sally Van Wert and O. C. Yoder, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Translational fusions were made between lacZ and the promoter regions of 3 *C. heterostrophus* genes: GPD1, TRP1, and PRO1. Each fusion was inserted as a single copy at the same chromosomal site in *C. heterostrophus* and assayed for expression under growth conditions that repressed endogenous β-galactosidase activity. The GPD1::lacZ fusion expressed 6 times more β-galactosidase activity and had a more abundant transcript than either the TRP1::lacZ or PRO1::lacZ fusion. These promoters will be useful to study the effects of altered virulence gene expression on host-parasite relationships of *C. heterostrophus*.

A286

MOLECULAR CHARACTERIZATION OF MITOCHONDRIAL GENOME IN CRYPHONECTRIA PARASITICA. N. Mahanti and D.W. Fulbright. Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

One hypovirulent strain of Cryphonectaria parasitica (CL25) from Michigan has all the characteristics of dsRNA associated hypovirulent strains but harbors no detectable amounts of dsRNA. Genetic studies with CL25 show that a cytoplasmic agent other than dsRNA might be involved in the CL25 type of hypovirulence. Restriction digest analysis of the mitochondrial genome of several virulent and hypovirulent strains using the restriction enzyme Sau3A showed that a band of about 2.5 kb is present in the CL25 strain but absent in other strains screened so far including CL1-16, a virulent strain from the same stand of trees in Michigan. This fragment has been cloned and the relationship of this RFLP fragment with the CL25 type of hypovirulence is under investigation.

A287

SEQUENCE HOMOLOGY BETWEEN FUNGAL DNA AND cDNA CLONES OF dsRNA IN CRYPHONECTRIA PARASITICA. C.M. Durbahn[1], D.L. Nuss[2] and D.W. Fulbright[1]. [1]Dept. Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824. [2]Dept. Molecular Oncology and Virology , Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

Although the presence of dsRNA molecules is closely correlated with hypovirulence in C. parasitica, little is known about the

role dsRNA plays in the reduction of fungal virulence. To elucidate this role, cDNA clones of dsRNA molecules found in C. parasitica strain GH2 were made. These cDNA clones were then used to probe Southern blots of total fungal DNA. Homology was detected between a cDNA clone of GH2 dsRNA and several C. parasitica strains, both virulent and hypovirulent. Using the polymerase chain reaction (PCR) technique, a product which shares homology with a GH2 cDNA clone was produced from the DNA of several strains. This PCR product has been cloned, and will be used as a probe to clone this entire GH2 region of homology from the fungal genome.

A288

MOLECULAR CHARACTERIZATION OF THE BETA-TUBULIN GENE FROM BENOMYL-SENSITIVE AND BENOMYL-RESISTANT FIELD STRAINS OF VENTURIA INAEQUALIS. H. Koenraadt, S.C. Somerville and A.L. Jones, Dept. of Botany and Plant Pathology and Pesticide Research Center, Michigan State University, East Lansing, MI 48824.

Benomyl-resistance in Venturia inaequalis has been attributed to mutations in the beta-tubulin gene. Field strains with differing levels of resistance provide a resource for characterizing the molecular changes responsible for the varying degrees of benomyl resistance. The beta-tubulin gene from a sensitive and a highly resistant isolate of V. inaequalis were cloned from genomic libraries using a Erysiphe graminis f. sp. hordei beta-tubulin probe. Sequence analysis showed that the V. inaequalis beta-tubulin gene has six introns and encodes a protein of 446 amino-acids. A comparison of the sequences of the beta-tubulin clones from the benomyl-sensitive and benomyl-resistant field strains will be presented.

A289

A DISPERSED, REPETITIVE DNA ELEMENT FINGERPRINTS MYCELIAL COMPATIBILITY GROUPS IN FIELD SAMPLES OF SCLEROTINIA SCLEROTIORUM. L.M. Kohn and J.B. Anderson. Dept. of Botany, University of Toronto, Erindale College, Mississauga, Ontario, L5L 1C6.

Sixty-four strains of Sclerotinia sclerotiorum were obtained from sclerotia from transects in two fields of Canola (oilseed rape) in Harriston, Ontario. Mycelial pairings of the strains in all combinations on agar medium produced either a compatible reaction in which two strains merged to form one uniform colony, or an incompatible reaction in which a reaction line developed in the interaction zone and the two strains, though growing together, remained distinct. Among the 34 strains of the first field, 6 mycelial compatibility groups (MCGs) were recognized, the largest group containing 19 strains. In the second field, 26 MCGs were recognized. Three molecular criteria indicated uniformity within MCGs; by one of these criteria, each MCG was uniquely fingerprinted. This fingerprint was produced by a random fragment of nuclear DNA (ca. 4.5 kb) from S. sclerotiorum, pLK44.20, that when used as a cloned probe in Southern blots of DNAs restricted with BamHI detected polymorphisms that corresponded exactly with strain groupings defined by mycelial compatibility. These data suggest that a field population of S. sclerotiorum is composed of genetically distinct "individuals".

A290

ANALYSIS OF ISOZYMES OF TWO ANASTOMOSIS GROUPS OF *RHIZOCTONIA SOLANI* ISOLATED FROM POTATO. J. Laroche, S. Jabaji-Hare and P. M. Charest. Dépt. de Phytologie, Université Laval, Ste-Foy, Québec, Canada, G1K 7P4.

Forty-eight isolates of *Rhizoctonia solani* belonging to anastomosis groups (AG) 3 and 9 and from different geographical regions were analyzed for isozyme polymorphism. The activity of 20 enzymes was screened using starch gel electrophoresis. Activity was detected for 7 enzymes, the scorable protein banding patterns of which were compared on polyacrylamide gel electrophoresis. The relative mobility values of electrophoretic bands were subjected to unweighted pair group mean average cluster analysis and principal coordinate analysis. Two major clusters were obtained matching the AG of 3 and 9 with some intra-group variation. Intra-group variation corresponded to the isolates' geographic origins. The resulting correlations between the present isolate grouping and the enzyme patterns reported here reinforce the anastomosis grouping concept and demonstrate that enzyme patterns constitute a useful and practical taxonomic tool.

A291

PROTOPLAST FORMATION AND TRANSFORMATION OF THE PHYTOPATHOGENIC FUNGUS *USTILAGO HORDEI*. K. E. Duncan and D. D. Pope, Department of Plant Pathology, University of Georgia, Athens, GA 30602.

Protoplasts of the barley smut fungus, *Ustilago hordei*, were generated by treating sporidia for one hour in a KCl buffer solution (0.6M KCl, 50mM MES) and the enzyme mixture NovoZym 234™ (20 mg/ml). Cell walls were enzymatically removed from sporidia with this treatment and eight percent of resulting protoplasts regenerated on Vogel's complete medium amended with 1M sorbitol. Protoplasts stabilized in buffer (40% PEG (w/v), 50mM $CaCl_2$, 10mM Tris-HCl (pH 7.5), 10mM EDTA) containing KCl (0.6M) were treated with pCM54 DNA, containing a gene for resistance to hygromycin. In one experiment, one hundred and forty-five putative transformants per μg of DNA were recovered after plating on medium amended with sorbitol (1M) and hygromycin (50 μg/ml). The transformation rate reported here is three- to fourteenfold higher than previously reported.

A292

MYCOPARASITISM OF SCLEROTIUM ROLFSII SCLEROTIA BY GLIOCLADIUM VIRENS. D. J. Collins and G. C. Papavizas. USDA-ARS, Biocontrol of Plant Diseases Laboratory, Beltsville, MD 20705.

Mycoparasitism of S. rolfsii sclerotia by G. virens was studied by light and scanning electron microscopy. To examine mycoparasitism an agar plug with mycelia of G. virens was placed in the center of a petri plate containing PDA, sclerotia of S. rolfsii was placed at the periphery of the plate and incubated for 1 week at 25 C. On PDA, G. virens inhibited mycelial growth from sclerotia and prevented formation of secondary sclerotia by S. rolfsii. Scanning electron micrographs of parasitized sclerotia showed extensive colonization and sporulation on the sclerotial surface and degradation of sclerotial walls. Cross sections of sclerotia, examined by light microscopy, revealed penetration of the sclerotial rind by hyphae of G. virens, and extensive internal colonization.

A293

TWO POTENTIAL MECHANISMS BY WHICH ATOXIGENIC STRAINS OF ASPERGILLUS FLAVUS PREVENT TOXIGENIC STRAINS FROM CONTAMINATING COTTONSEED. P. J. Cotty, P. Bayman, and D. Bhatnagar. USDA, ARS, Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA 70124.

Atoxigenic strains of Aspergillus flavus can reduce aflatoxin production by toxigenic strains when developing cotton bolls are inoculated simultaneously with both strains. Relative abilities of toxigenic and atoxigenic strains to infect and colonize developing seed were demonstrated with mutants unable to reduce nitrate. Results suggest that competitive exclusion may partially explain the efficacy of atoxigenic strains. An atoxigenic strain inhibited toxin production by toxigenic strains similarly in vitro and in vivo. However, it did not inhibit the growth of the toxigenic strain on agar plates. The atoxigenic strain reduced toxin production even when mycelial balls of the two strains were grown independently for 24 hrs prior to co-fermentation. Thus, direct inhibition of toxin production may be a second mechanism by which atoxigenic strains prevent contamination.

A294

CONTROL OF SOFT ROT ERWINIAS WITH BACTERIOPHAGES. Cynthia G. Eayre, Diane E. Concelmo, and J.A. Bartz, Plant Pathology Dept., University of Florida, Gainesville, Fl 32611.

Bacteriophages of Erwinia carotovora subsp. carotovora were isolated from lake water by a standard enrichment technique. At least 16 different phages were identified based on host range against 71 strains of soft rot erwinias. One phage caused plaques in lawns of at least one strain of each of the three major soft rot erwinas, e.g., E. c. atroseptica, E. c. carotovora, and E. chrysanthemi. Certain phages infected up to 65% of the strains of E. c. carotovora. However, none of the phages caused plaques in lawns of E. herbicola. Bacterial soft rot in slices of potato tubers was prevented when 12 μl of 10^6 cfu E. c. carotovora/ml. When the concentration of the inoculum was 10-fold greater, some decay occurred, but lesion diameters were reduced by the bacteriophage.

A295

THE STIMULATION OF PYTHIUM ULTIMUM BY SEED VOLATILES AND THE INTERACTION OF PSEUDOMONAS PUTIDA. T. C. Paulitz, Dept. of Plant Sciences, Macdonald College of McGill University, Ste. Anne de Bellevue, Quebec, Canada H9X 1C0.

Pseudomonas putida strain N1R, applied to seeds, increased emergence of soybean and pea in soil infested with Pythium ultimum. Germination and hyphal growth of soil-produced sporang-

gia were stimulated by volatiles from imbibed seeds of pea and soybean, but stimulation was reduced when seeds were treated with N1R. Hyphal growth of inoculum was not reduced by exposure to volatiles from N1R growing on King's medium B agar. The concentration of ammonium/ammonia was similar in the spermosphere of N1R-treated and non-treated seeds. The concentration of ethanol and acetaldehyde produced by pea seeds, and acetaldehyde produced by soybean seeds were reduced by treatment with N1R. Hyphal growth of sporangial inoculum was stimulated by exposure to ethanol at 3-10 ul/ml. This suggests that N1R antagonizes P. ultimum by interfering with seed volatile-induced germination and growth, rather than by producing volatile antimicrobial compounds.

A296

POTENTIAL OF GLIOCLADIUM ROSEUM AND G. CATENULATUM FOR BIOCONTROL OF VERTICILLIUM DAHLIAE. A. P. Keinath, D. R. Fravel, and G. C. Papavizas, USDA, ARS, Beltsville, MD 20705.

Vermiculite:wheat bran (3:1 w/w) colonized by G. roseum or G. catenulatum was added to nonsterile soil at rates $\leq$ 0.1% w/w. Microsclerotia of V. dahliae embedded in nylon mesh squares were buried in amended soil, recovered after 1, 2, 6, 10 or 14 days and plated on soil extract-polygalacturonic acid agar to assess viability. G. roseum reduced viability of V. dahliae by 80-90% even after 1 day of incubation. Six of seven isolates of G. roseum and both isolates of G. catenulatum used were effective for biocontrol at rates of 0.01-0.05% antagonist preparation in soil ($P \leq 0.05$). Alginate pellets were formulated with either vermiculite:bran colonized by G. roseum or bran plus a conidial suspension. Although colony-forming units/g pellet were 2-200 times greater with vermiculite:bran than with the conidial suspension, only pellets formulated with the conidial suspension were effective. This information is being used for field application of G. roseum.

A297

INFLUENCE OF SOIL EDAPHIC FACTORS ON SUPPRESSION OF TAKE-ALL BY PSEUDOMONAS FLUORESCENS 2-79. B.H. Ownley, D.M. Weller, and J.R. Alldredge. USDA, ARS, Washington State University, Pullman, WA 99164-6430.

Pseudomonas fluorescens 2-79 suppresses take-all of wheat caused by Gaeumannomyces graminis var. tritici, but the level of disease suppression is variable from site to site. To assess the relative importance of soil edaphic factors on take-all suppression, seeds were treated with 2-79 (produces phenazine-1-carboxylic acid, Phz^+), Phz^- mutants, or mutants genetically restored to the Phz^+ phenotype, and sown in ten different steamed soils. Disease suppression by Phz^+ strains was positively correlated with ($P=0.01$) sulfate-sulfur, % sand; ($P=0.05$) pH, sodium, zinc, and ($P=0.20$) ammonium-nitrogen. Suppression was negatively correlated with ($P=0.05$) cation exchange capacity, exchangeable acidity, manganese, iron, % silt, % clay, and ($P=0.20$) % organic matter and total carbon. Regression models ($R^2 > 0.95$) for take-all suppression by Phz^+ strains included the soil characteristics: cation exchange capacity, sulfate-sulfur, magnesium, potassium, sodium, ammonium-nitrogen, total nitrogen, % organic matter, total carbon, % sand, copper, pH, zinc, and % silt.

A298

BACTERIAL COMMUNITIES IN SOIL AND ON SOYBEAN ROOTS, AND THE EFFECTS OF A BIOLOGICAL CONTROL AGENT. G. S. Gilbert, J. Handelsman, and J. L. Parke. Dept. of Plant Pathology, Univ. of Wisconsin-Madison, WI 53706

Communities of bacteria in bulk soil, on untreated soybean roots, and on roots from soybean seed treated with the biocontrol agent *Bacillus cereus* strain UW85n were distinguished by physiological attributes of the bacteria. The plants were grown both in the field and in a growth chamber with sieved, air-dried soil from the field site. Approximately 200 aerobic bacteria from each habitat were randomly selected from 10% trypticase soy agar. Each isolate was tested for a wide range of physiological characteristics, including extracellular enzyme activity, growth on single carbon sources, anaerobic growth, motility, and resistance to antibiotics and salts. Multivariate analyses of the physiological attributes of the field isolates showed that the three habitats contained distinct bacterial communities. Resistance to certain antibiotics was more prevalent on both treated and untreated roots than in the soil. Pectolytic activity was most common among isolates from the UW85n-treated roots. More soil isolates than root isolates produced various extracellular enzymes. Communities from the growth chamber experiment resembled those from the field in some respects but not others. Our results indicate that the introduction of a single organism for control of plant disease can have a significant impact on plant-associated bacterial communities.

A299

ROLE OF RHIZOSPHERE COMPETENCE ON ANTAGONISTIC ACTIVITY OF SAPROPHYTIC FUSARIA ISOLATED FROM SUPPRESSIVE SOILS. A.Garibaldi and M.L.Gullino, Istituto di Patologia vegetale, Via Giuria 15, Torino, Italy.

Saprophytic Fusaria, isolated from rhizosphere of plants grown in soils suppressive to several formae speciales of pathogenic Fusarium oxysporum, actively colonized rhizosphere of different hosts in steamed and raw soils. Fusaria densities were significantly higher at the root tip and at the base of the stem than in the midportion of the root. Highly rhizosphere competent Fusaria showed the best activity against carnation wilt when applied, at transplanting, by dipping rooted cuttings. Integration of dipping roots of rooted cuttings in conidial suspensions of benzimidazole resistant antagonistic Fusaria with soil application of benomyl (5-10 g/m^2) improved wilt control. Use of these rhizosphere competent Fusaria eliminates the need of adding large amounts of inoculum to soil in order to induce suppressiveness.

A300

Glucose Oxidase-Producing and Non-producing Strains of Talaromyces flavus for Biocontrol in Verticillium Wilt of Eggplant. D. R. Fravel and D. R. Roberts, USDA, ARS, Beltsville, MD 20705.

An isolate (Tfl-np) which produced 45 times less glucose oxidase than the wild-type T. flavus Tfl was selected by screening natural variants of Tfl. In a greenhouse test in nonsterile field soil, Tfl controlled Verticillium wilt of eggplant while Tfl-np did not. No differences were found when the two isolates were compared for conidial or ascospore production, recovery from nonsterile field soil, or growth rate on agar. Comparison of extracellular protein profiles between the two isolates by SDS-polyacrylamide gel electrophoresis also showed that Tfl-np produced substantially less glucose oxidase than Tfl. In addition, there were differences in quantity of other proteins produced. These data suggest the in situ involvement of glucose oxidase in biocontrol of Verticillium wilt of eggplant; however, other proteins may also be involved.

A303

THE ROLES OF INDIGENOUS FUSARIUM OXYSPORUM AND VARIOUS OTHER MICROORGANISMS IN A SOIL SUPPRESSIVE TO FUSARIUM WILT

OF WATERMELON. R.P. Larkin, D.L. Hopkins, and F.N. Martin, Dept. of Plant Pathology, University of Florida, Gainesville, FL 32611.

Suppressiveness to Fusarium wilt of watermelon developed through monoculture to cv.'Crimson Sweet' (Phytopath.77:607–611) was destroyed in soil exposed to microwave (MW) irradiation (2450 MHz, 700 watts) for 90s/kg soil at −0.01 MPa matric potential. This treatment eliminated F. oxysporum and most other fungi from the soil, but had only a slight effect on total numbers of bacteria and actinomycetes. Over 100 isolates of F. oxysporum and 250 isolates of miscellaneous bacteria, actinomycetes, and fungi isolated from the roots of watermelon plants grown in suppressive or conducive soils were tested for their ability to restore suppressiveness to a MW–treated soil. Successful isolates were also tested singly and in combination in a conducive field soil. Several isolates of F. oxysporum were most successful in reducing wilt (35–75% reduction) in both soils. Root colonization characteristics of selected isolates and mechanisms of suppression were also investigated.

A304

INCREASE OF TRICHODERMA POPULATIONS IN SOIL ASSOCIATED WITH ADDED RHIZOCTONIA SOLANI HYPHAE. M. E. de la Fuente and C. A. Martinson, Plant Pathology, Iowa State University, Ames,IA 50011

Rhizoctonia solani was grown on sterile beet seed; soil was amended with these infested seed (50/300g soil), seed sterilized with propylene oxide to kill R. solani, uninfested sterile seed, or no seed. Soil was infested with T. viride spores to 2, 4, and 8x the natural population. Trichoderma populations increased to $1x10^6$ in 4 days in soil amended with living R. solani and with no added T. viride (1x), higher than in soils infested with T. viride (2, 4, 8x). Seed with killed R. solani hyphae and sterile seed had little effect on Trichoderma populations. Seed with living R. solani hyphae were excellent baits for isolation of Trichoderma from soil, even at very low populations. In soil repeatedly cropped to radish, wheat, or cucumber or uncropped, Trichoderma populations increased significantly only when R. solani was infested into the soils; with added R. solani inoculum, Trichoderma populations increased from $3x10^3$ to $2x10^6$. Viable R. solani inocula stimulate Trichoderma activity in soil.

A305

TRANSFER OF HERBICIDE PRODUCTION GENES FROM STREPTOMYCES HYGROSCOPICUS INTO A PLANT PATHOGEN, XANTHOMONAS CAMPESTRIS pv. CAMPESTRIS. Virginia Joan Prange and R. Charudattan, Plant Pathology Dept., University of Florida, Gainesville, 32611.

Genes encoding the production of bialaphos, a potent glutamine synthetase inhibitor, have been cloned into a plant pathogen, Xanthomonas campestris pv. campestris (XCC), in order to create an experimental model to study alterations in pathogenic traits. These genes were originally isolated from Streptomyces hygroscopicus (ATCC 21705) and cloned into cosmid vector pBG9 (Murakami et. al, 1986, Mol. Gen. Genet. 205:42–50). We have transferred these genes into pLAFR3, a cosmid functioning in both E. coli and XCC. The resulting cosmid, named pIL–1, was transformed into E. coli and incorporated into XCC by conjugation. Characterization of pIL–1 with regard to cosmid maintenance, generation time, and bioactivity detected in plant and microbial assays will be presented.

A306

A MODEL FOR THE EXPANSION OF COHORTS OF LESIONS ON COHORTS OF LEAVES. R. D. Berger and A. Bergamin, Departments of Plant Pathology, University of Florida, Gainesville, FL 32611 and University of Sao Paulo/ESALQ, Piracicaba, Brazil 13400.

Six epidemiological parameters were incorporated into a modification of Vanderplank's basic-infection-rate equation to obtain a model for disease progress with expansion of lesions. Daily cohorts of infections were initiated on age-defined cohorts of host area. A radial increase of >0.05 mm/day for the circular lesions resulted in >85% of the total disease being from lesion expansion. Expansion of lesions was an especially important component of total disease as latent period lengthened beyond 5 days. As the percentage of hypothetical susceptible sites increased above 1%, lesion expansion became a less important component of total disease; while the percentage of lesion area with sporulation, the maximum basic infection rate, and initial lesion size were relatively insensitive parameters. Natural epidemics of Cercospora and Lepto spots and rust of alfalfa and early blight of potato were used to validate the model.

A308

RELATIONSHIP OF EPIPHYTIC POPULATIONS OF CITRUS BACTERIAL SPOT BACTERIAL STRAINS TO DISEASE DEVELOPMENT AND SURVIVAL. T. R. Gottwald, J. H. Graham, USDA/ARS, Orlando, FL 32803.

One end of each of five citrus nurseries was inoculated with one of three bacterial strains of Xanthomonas campestris pv citrumelo (Xcc) representing aggressive (AG), moderately aggressive (MAG), and weakly aggressive (WAG) isolates of Xcc. Blowing rainstorms were simulated with a mist blower by spraying water down rows at high velocity over the inoculated plants toward receptor plants. Epiphytic populations of Xcc, sampled on receptor plants on day 1, correlated well (r = 0.679 to 0.960) with disease severity of these same plants 1, 20, 40 and 76 da following the event. Slopes of the bacterial dispersal gradients were directly related to eventual disease development and strain aggressiveness. Disease severity (DS) decreased in all nurseries over time at ca. the same rate. Survival of Xcc strains was tested under simulated citrus grove conditions. Disease decrease was nearly linear for each strain with slopes of r = −0.0054, −0.0061, −0.0067 DS/da for Swingle and r = −0.0067, −0.0055, −0.0018 DS/da for grapefruit for AG, MAG, and WAG strains, respectively.

A309

EPIDEMIOLOGY OF BIOLOGICAL CONTROL OF APHANOMYCES ROOT ROT OF PEA AND PYTHIUM PRE-EMERGENCE DAMPING-OFF. J. H. Bowers and J. L. Parke, Dept. Plant Pathology, University of Wisconsin, Madison, 53706.

Field plots were established to investigate the efficacy of seed treatments with *Pseudomonas fluorescens* strain PRA25, *P. cepacia* strain AMMD, *Corynebacterium* spp. strain 5A, and captan on the epidemiology of root rot of pea caused by *Aphanomyces euteiches*. Emergence was evaluated 19 days after planting and disease incidence was determined every 2 to 3 days thereafter until harvest. Pre-emergence damping-off, caused by *Pythium* spp., was affected by the treatments with the bacterial strains having significantly greater emergence (P=.05) than the captan treatment. To determine the effect of treatments on disease progress following emergence, piecewise regression was used in which the post-emergence epidemic was divided into two halves. The change in the rates of disease progress was associated with changes in soil temperature and soil-water matric potential. The rates of symptom expression among treatments were not significantly different within the first or second half of the epidemic, except that the AMMD treatment had a significantly higher rate of symptom expression in the first half of the epidemic even though disease incidence was lower. Each bacterial treatment was also found to have significantly less area under the disease progress curve (AUDPC) and significantly less disease severity at 10% bloom than the captan treatment. Although the analyses indicated that disease progressed at similar rates regardless of the seed treatment and that the level of disease incidence may be determined by the effectiveness of the treatments against pre-emergence damping-off, the differences in disease severity indicate that *A. euteiches* is also controlled by the bacterial treatments.

A310

AN EXPANDED PARALOGISTIC MODEL OF RICE LEAF BLAST. Paul S. Teng and Jonathan E. Yuen, respectively, International Rice Research Institute, P.O. Box 933 Manila, The Philippines, and University of Hawaii, 3190 Maile Way, Honolulu, HI 96822, U.S.A.

The paralogistic equation of J.E. VanderPlank (1963), $dx_t/dt = R_c \cdot (x_{t-p} - x_{t-i-p}) \cdot (1-x_t)$ was numericaly simulated using a program called STELLA™ (High Performance Systems, New Hampshire) on an APPLE™ Macintosh microcomputer. The *Equivalence Theorem* was tested by incrementally building weather stochasticity (temperature, RH and rainfall) and biological relationships (latent period, infectious period, infectivity, receptivity) into the paralogistic. Stochastic weather was generated using WGEN developed by Richardson and Wright (1984). Epidemics of rice leaf blast caused by *Pyricularia oryzae* were simulated for Asian sites ranging from cool temperate to hot, tropical. The blast epidemiologic potentials of different locations were calculated and then compared using stochastic dominance. This technique has potential use in guiding rice genotype deployment .

A311

COUPLING PEST EFFECTS TO THE IBSNAT CERES CROP MODEL FOR RICE. H. Pinnschmidt, Dept. Agron. and Soil Sci., P.S. Teng, J.E. Yuen, Dept. Plant Pathol., Univ. of Hawaii at Manoa, Honolulu, Hawaii 96822, and A. Djurle, Swedish Agric. Univ., Dept. Plant Pathol., 75007 Uppsala, Sweden.

An approach to link population models for leaf blast, panicle blast, sheath blight, leaf folder, stem borers, and plant hoppers to the IBSNAT CERES rice model was developed. Development of each disease or pest was modeled using a generic type model, derived from a paralogistic growth function. Pest effects were introduced into the crop simulator by mimicking effects at the physiological process level, following the "coupling point" concept of BOOTE et al. (1983). The physiological processes and crop variables affected were: light use efficiency, photosynthesis, partitioning, amount and translocation of carbohydrates, evapotranspiration, leaf area index, stand density, senescence rate, grain filling, and panicle weight. Crop variables, such as leaf area index and stand density served as driving variables for pest development resulting in a method of handling pest competition.

A312

MICROCLIMATES IN TWO CROPPING SYSTEMS AND THE RELATIONSHIP TO INTENSITY OF ANGULAR LEAF SPOT OF BEAN. J. M. Lanter, J. G. Hancock, and J. W. White*. Dept. of Plant Pathology, University of California, Berkeley, CA 94720 and *CIAT, AA6703 Cali, Colombia.

Several microclimatic variables were continuously monitored and recorded in bean monocultures and bean-maize intercrops during three field trials (two rainy seasons, one dry season) in 1986-87 at the International Center for Tropical Agriculture. Temperature in- and outside the canopy, vapor pressure, saturation deficit, and duration of leaf wetness were compared by discriminant analysis. Significant canonical correlations of 0.834, 0.722, and 0.768 were found for the three trials when hourly data for the entire season were analyzed, and correlations improved as subsets of the data were analyzed separately. No single variable was consistently most important in distinguishing between treatments; however, duration of leaf wetness was the only variable that coincided with intensity of angular leaf spot (ALS). In each trial, the treatment with the longer duration of leaf wetness had the greatest ALS intensity. During the rainy seasons, monocultures averaged 0.84 and 1.48 hr/day longer with condensed moisture than intercrops, whereas during the dry season, the intercrop averaged 0.59 hr/day longer with condensed moisture than the monoculture.

A316

ASSESSING RISK WITH STOCHASTIC DOMINANCE. Jonathan E. Yuen and Paul S. Teng, respectively, University of Hawaii, 3190 Maile Way, Honolulu, HI 96822 USA and the International Rice Research Institute, PO Box 933, Manila, The Philippines.

The riskiness of different cropping strategies was assessed using stochastic dominance (Hadar and Russell, 1969; Meyer, 1977). A disease simulation model for potato late blight (Bruhn and Fry, 1981) was used with both simulated and historical weather data files to calculate net return from cropping strategies with different disease control measures. Probability density functions (pdf's) for the net return resulting from different disease control measures were then estimated. These pdf's were compared using first and second degree stochastic dominance. First degree stochastic dominance identified strategies with low return and which would not have been chosen by decision makers seeking to maximize return. Second degree stochastic dominance eliminated disease control strategies that would not have been chosen by decision makers that are risk-averse. Stochastic dominance has the ability to compare pdf's without knowing the preferences of the decision maker.

A317

EFFECTS OF CLOVER YELLOW VEIN VIRUS ON EPIDEMIC COMPONENTS OF CERCOSPORA LEAF SPOT ON WHITE CLOVER. Scot C. Nelson and C. Lee Campbell. Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Epidemic components of leaf spot caused by Cercospora zebrina were assessed on two clones of Trifolium repens that vary in response to infection by clover yellow vein virus (CYVV). Healthy and CYVV-infected plants of clones T7 (low virus titer, asymptomatic) and T17 (high virus titer, symptomatic) were grown at 28/23 C or 22/17 C and monitored for 17 days after inoculation with C. zebrina. Alterations of epidemic components for CYVV-infected plants of T17 were: diminished infection efficiency, shortened latent period, larger lesions, greater proportion of leaves with sporulating lesions, and reduced defoliation, disease incidence and severity. Incubation period (28/23 C) and latent period were shortened for CYVV-infected plants of T7. A clone x virus x temperature interaction was found for incubation period and lesion diameter. Incidence of infected leaves was greater at 22/17 C, regardless of clone/virus combination.

A318

PATTERNS OF ASSOCIATION AMONG PATHOGENS ON LEAFLETS OF ALFALFA. Jim A. Duthie and C. Lee Campbell, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616.

Presence of five pathogens on each of 5,800 diseased leaflets of Raidor alfalfa from 19 sampling dates over 2 yr was recorded. For each of 10 pairwise combinations, strength of association between two pathogens was estimated by ln(A/B) where A was the odds that one pathogen was present given that the second pathogen was present, and B was the odds that the first was present given that the second was absent. On most dates, strong positive associations between Leptosphaerulina trifolii and Stemphylium botryosum and between Cercospora medicaginis and Colletotrichum spp. were detected. Associations between L. trifolii or S. botryosum and C. medicaginis or Colletotrichum spp. usually were weak or negative. Phoma medicaginis often was associated negatively with L. trifolii and positively with Colletotrichum spp. Pathogens occurred in two distinct complexes, one of L. trifolii and S. botryosum and another of P. medicaginis, C. medicaginis, and Colletotrichum spp.

A319

QUANTIFICATION OF DISEASE PROGRESS AND DEFOLIATION IN THE *POPULUS DELTOIDES-MELAMPSORA MEDUSAE* PATHOSYSTEM. R. C. Hamelin, L. Shain, B. A. Thielges* and R. S. Ferriss. Departments of Plant Pathology and Forestry*, University of Kentucky, Lexington, KY 40546-0091.

Disease progress and defoliation were quantified in 11 half-sib families derived from natural stands of *P. deltoides*. Values for the area under the disease progress curve (AUDPC), the shape (m) and absolute rate (Ra) parameters derived from the Richards growth model and the final disease severity (Yf) ranged from 0.42- 4.32, 0.97- 3.9, 0.0012- 0.009 and 0.06-0.38, respectively. Values for the number of days before leaf fall (DLF) and the area under the leaf area curve (AULAC) ranged from 50.02-66.58 and 4.52-7.15, respectively. The AUDPC was strongly correlated with DLF ($r = -0.86$), Yf ($r = 0.96$) and Ra ($r = 0.84$); strong correlations also were found between DLF and Yf ($r = -0.94$) and DLF and Ra ($r = -0.84$). These results confirm the causality of *M. medusae* on early defoliation of *P. deltoides*. A cluster analysis grouped families into 2 clusters: cluster 1 was composed of families from the northernmost locations and had larger AUDPC, Yf and Ra, and lower DLF and AULAC than cluster 2 which was comprised of families from the southermost locations, indicating higher rust susceptibility in northern than southern families in the U.S. at the test site in Carlisle Co. KY.

A321

SEED TRANSMISSION AND ADDITIONAL HOST PLANT OF A VIROID FROM COLEUS. R.P. Singh, A. Boucher, and A. Singh, Agriculture Canada, Research Station, P.O. Box 20280, Fredericton, N.B., Canada E3B 4Z7.

A survey for viroids in 18 ornamental plant species was made. Nucleic acids were extracted, purified further by CF-11 column chromatography and analyzed for viroid-like RNA by return-polyacrylamide gel electrophoresis (R-PAGE). Viroid-like RNAs were detected from plants of Coleus (Coleus scutellaroides) and goldfish (Hypocyrta mummularia). The viroid from Coleus was transmitted to additional Coleus seedlings and Ocimum sanctum plants by mechanical and graft-inoculation. It was carried symptomlessly in both hosts, and migrated on gel in a manner similar to yellow Coleus viroid reported from Brazil. Using R-PAGE, viroids were detected occasionally from single Coleus seeds weighing ca. 325 ug, and routinely from a group of 3 seeds/sample. Viroids were detected in a high percentage (ca. 70%) of seedlings grown from seeds of infected plants. Initially, viroids were detected in Coleus plants grown from seeds imported from South America. The viroids could have been carried in the infected seeds and subsequently infected other plants.

A322

LONG-TERM PERSISTENCE OF POTATO SPINDLE TUBER VIROID IN TRUE POTATO SEEDS (TPS). R.P. Singh, A. Boucher, and R.G. Wong, Agriculture Canada, Research Station, P.O. Box 20280, Fredericton, N.B., Canada E3B 4Z7, and Keshan Potato Research Institute, Heilongjiang, Peoples Republic of China.

Return-polyacrylamide gel electrophoresis (R-PAGE) was applied to true potato seeds (TPS) obtained from the potato breeding program of the Keshan Potato Research Institute, China, for the detection of potato spindle tuber viroid (PSTVd). Sixty-five percent of the 36 crosses tested, made before 1985, contained PSTVd. The viroid was detected in similar percentages whether the TPS was from inbred lines or from out crosses. TPS stored in paper bags at room temperature as far back as 1965 were also tested for PSTVd. The viroid was detected from TPS stored for 21 years. Inocula of dormant TPS prepared either as sap or nucleic acid extracts were equally infectious to tomato plants. The viroid concentration in tomato plants inoculated with both extracts attained similar levels within four weeks of inoculation.

A323

DETECTION OF TOMATO SPOTTED WILT VIRUS RNA IN THRIPS USING STRAND SPECIFIC PROBES. Thomas L. German and Yi Hu. Department of Plant Pathology, University of Wisconsin, Madison, 53706 and University of Hawaii, Manoa, 96822.

We have constructed a library of cDNA clones in the plasmid vector PBR322 and used these to develop a diagnostic dot blot assay for tomato spotted wilt virus (TSWV). This assay will detect viral RNA in 16 ng of total RNA and is useful for detecting the presence of the virus in all hosts tested and in individual thrips (Rice et al, Plant Disease, in press). One of these clones (pTSWV80) was subcloned into the vector Blue Script II to take advantage of the T7 and T3 strand specific promoters. Probes were generated and used to assay for the presence of each RNA strand in individual plant and insect samples. We were able to determine a time course for appearance of each strand in plants and to demonstrate the presence of both strands in thrips. Although we have not assigned polarity with respect to translation of the strands, these data indicate that both (+) and (-) sense RNAs occur in insects.

A324

TEMPORAL ACCUMULATION OF CAULIFLOWER MOSAIC VIRUS DNA, RNA, AND COAT PROTEIN IN RELATION TO SYMPTOM SEVERITY IN TURNIPS. E. J. Anderson, A. T. Trese, and J. E. Schoelz, Dept. of Plant Pathology, University of Missouri, Columbia, Missouri 65211.

Cauliflower Mosaic Virus (CaMV) strains W260 and CM1841 produce markedly different symptoms when inoculated onto turnips (Brassica campestris L. 'Just Right'). Strain W260 causes a prominent mosaic accompanied by moderate to severe stunting of both leaves and turnips, while infection by CM1841 results in a prominent mosaic and only mild stunting. As part of our effort to correlate viral gene function with symptom development we have used ELISA and DNA dot-blot hybridization to measured viral coat protein and DNA accumulation in W260- or CM1841-infected leaves. The inoculated, 1st, 2nd, 5th, and 10th systemically infected leaves were assayed independently, once available, from 0 through 56 days postinoculation. While CM1841 caused significantly more coat protein accumulation than did W260, only a slight increase in viral DNA accumulation was observed. Further experiments are in progress to determine whether increased accumulation of CM1841 coat protein is due to increased synthesis of the 35S RNA.

A325

EVIDENCE FOR GENETIC RECOMBINATION IN THE 5' END OF THE COAT PROTEIN GENE OF A STRAIN OF SUGARCANE MOSAIC VIRUS. D.D. Shukla, M.J. Frenkel, N.M. McKern, P.M. Strike and C.W. Ward, CSIRO, Division of Biomolecular Engineering, Parkville, Victoria, 3052, Australia.

The 3'terminal 1343 nucleotides of the SC strain of the sugarcane mosaic virus (SCMV-SC) genome have been compared with the sequence of maize dwarf mosaic virus B (MDMV-B). The coat protein of SCMV-SC shows high sequence identity (92%) with that of MDMV-B except for the region between amino acid residues 27 and 70. This region is smaller (44 residues) than the equivalent region in MDMV-B (59 residues) and shows low sequence identity (22%) to the MDMV-B sequence. The sequence diversity in this region indicates the occurrence of a recombination event during the evolution of the virus. Sequence identities of both the major part of the coat proteins and the non-coding regions confirm that SCMV-SC and MDMV-B are strains of SCMV.

A326

ANALYSIS OF THE CARLAVIRUS ASSOCIATED WITH SHEEP PEN HILL DISEASE OF BLUEBERRIES. **T.D.Cavileer, B.T.Halpern, and B.I.Hillman, Dept. of Plant Pathology, Rutgers University, New Brunswick, NJ 08903.**

A Carlavirus is associated with Sheep Pen Hill disease (SPHDAV) of blueberries in New Jersey, and a similar Carlavirus causes blueberry scorch disease in the Pacific Northwest. Beginning with RNA extracted from virus that was isolated from blueberry tissue and from mechanically infected Chenopodium quinoa plants, we synthesized cDNA libraries representing the genome of SPHDAV. Many of the cDNA clones from a library that was initiated by priming with oligo d(T) contained a poly (A) tract followed by residues that were identical for all clones examined, identifying this as the 3' terminus. The sequence for approximately 2 kb at the 3' terminus has been determined and compared with published sequences for the Carlaviruses PVM and PVS. There was extensive conservation among the three genomes, and they appear to be organized similarly.

A327

SOMACLONAL VARIATION FOR RESISTANCE TO SHEATH BLIGHT IN RICE. Q.J. Xie, M.C. Rush, and J. Cao. Dept. Plant Pathology and Crop Physiology, LA Agr. Expt. Sta., Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

Variation in resistance to rice sheath blight was observed in somaclones regenerated from the susceptible long-grain cultivar Labelle. Among 2,100 R2 somaclonal lines inoculated and screened for sheath blight resistance, three lines regenerated from this cultivar showed a high level of resistance. The sheath blight resistant somaclone SC 86-20001 has good agronomic characteristics when compared to the previously available resistant cultivars. The resistance of the three somaclonal lines to sheath blight was stable after 4 years of tests in the field and in the greenhouse. The inheritance of sheath blight resistance in SC 86-20001-5 was controlled by a single recessive gene. Sheath blight resistance of SC 86-20001-33 was controlled by two independently inherited recessive genes. Selection among F3 lines from crosses of the above lines with the cultivar Lemont gave lines with resistance, good plant type, and high yield potential.

A328

ADAPTATION OF *PUCCINIA RECONDITA* TO SLOW-RUSTING WHEAT CULTIVARS DUE TO ARTIFICIAL SELECTION. J.S. Lehman and G.E. Shaner, Botany & Plant Pathology Dept., Purdue University, W. Lafayette, IN. 47907

To study adaptation of *Puccinia recondita* to slow-rusting wheat cultivars, a wild-type population (WT) was reared and selected for reduced latent period (LP) for five generations on slow-ruster CI 13227. WT, first (C1), and fifth (C5) generation isolates were evaluated on fast-rusting cultivar Monon and slow-rusting cultivars CI 13227, L574, Suwon 85, and SW 72469-6 for differences in LP (greenhouse) and disease levels (field). C5 had a shorter LP and higher disease levels than WT. C1 did not differ from WT. CI 13227 and Suwon 85 had significantly longer LPs than Monon, while L574 and SW 72469-6 had intermediate values. Field comparisons of cultivars were less clear. Disease levels on L574 and Monon were similar despite L574 having a significantly longer LP. Also, SW 72469-6, with a shorter LP than CI 13227 and Suwon 85, had the least amount of disease. A significant cultivar X isolate interaction was detectable in the greenhouse and was mainly due to C5 on CI 13227. In the field, no interaction existed, but C1 and C5 on CI 13227, and C5 on L574, did differ significantly from WT on these cultivars. These results suggest isolate adaptation and some degree of race-specificity toward slow-rusting resistance, but not sufficient to overcome the resistance.

A331

SEEDLING SCREENING PROCEDURES FOR DETECTING RESISTANCE TO BACTERIAL SPOT (Xanthomonas campestris pv. vesicatoria) OF TOMATO. G.C. Somodi, J.B. Jones, J.W. Scott, and J.P. Jones. GCREC, 5007 60th Street E., Bradenton, FL 34203.

An adaptation of a cotyledon-dip inoculation (CDI) technique developed for bacterial speck (Pseudomonas syringae pv.tomato) resistance screening was compared with other potential bacterial spot (BS) seedling inoculation procedures. Inoculum concentration (10^6 or 10^8 cfu/ml), plant age (1, 2, or 3 weeks) and pre-inoculation regimes (dry or water-congested tissue), were tested for identifying susceptible and resistant genotypes. Comparisons were made to field BS ratings to determine which seedling screen differentiated genotypes similarly. Two or 3 week old plants with water-congested tissue (held at 100% RH for 16h) prior to inoculation with 10^8 cfu/ml, followed by incubation in a growth chamber at 28C and high RH, resulted in highly significant correlations (0.83-0.97) with field ratings whereas disease ratings for other treatments or those treated by the CDI procedure generally were not significantly correlated with field results.

A332

VARIATION AMONG FIRST GENERATION SOMACLONES AND IRRADIATED TOMATO SEEDLINGS IN RESPONSE TO CLAVIBACTER MICHIGANENSIS SUBSP. MICHIGANENSIS. R.M. De Vries-Paterson and C.T. Stephens. Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824-1312.

First generation progeny of (a) 283 somaclones regenerated from leaf explants of 12 Lycopersicon cvs. and (b) 53 irradiated L. esculentum (2 cvs.) lines were analyzed for resistance to Clavibacter michiganensis subsp. michiganensis (Cmm), as measured by foliar symptoms and pathogen populations. Disease resistance varied among clones. Both susceptible and partially resistant cvs. exhibited significant increases in Cmm resistance as the result of somaclonal variation (SV). SV was found to be more efficient (quantitatively) at increasing resistance to Cmm, compared to induced mutation by gamma irradiation. One irradiated (20 kilorad) 'Sunny' line exhibited a significant increase in Cmm resistance. The nature of Cmm resistance was not generally expressed by a reduced pathogen population. This is the first report of two types of induced mutations both resulting in increased resistance to Cmm in tomato.

A333

RELATIVE RESISTANCE OF SOUTHERN RUNNER PEANUT TO STEM ROT IN COMPARISON TO OTHER CULTIVARS. T. B. Brenneman[1], W. D. Branch[2], and A. S. Csinos[1], Departments of Plant Pathology[1] and Agronomy[2], UGA, Coastal Plain Experiment Station, Tifton, GA 31793

The susceptibility of 16 peanut (Arachis hypogaea L.) genotypes (eight virginia and eight runner types) to southern stem rot (Sclerotium rolfsii Sacc.) was evaluated in the field for three years. The mean yield for the eight virginia types was 3287 kg/ha versus 3214 for the eight runner types. The mean disease incidence was 14.3 disease loci per 12.2 m of row for both market types. Of the virginia types, NC 6 and Florigiant were the most susceptible with NC 9, VA 81B and Early Bunch being the most resistant. Most runner cultivars were quite susceptible except for Southern Runner and Langley which had about 50% less disease than the most susceptible entries. Southern Runner had the lowest disease incidence and highest pod yield of any cultivar. Compared to Florunner, the current industry standard, it had about 50% less disease and yields were 1346 kg/ha higher. There was a highly significant negative correlation ($P = 0.01$) between yields and disease incidence all three years.

A334

RESISTANCE TO KEY DISEASES IN SUB-SPECIFIC GROUPS OF RICE. J.M. Bonman, T.W. Mew, H. Koganezawa, T.I. Vergel de Dios-Mew, C.M. Vera Cruz, E.S. Medalla, C.K. Kim, J.-L. Notteghem, J.-C. Glaszmann. IRRI, P.O. Box 933, Manila, Philippines; IAS, Rural Dev., Adm., Suweon, Korea; CIRAD/IRAT, B.P. 5035, France.

From the International Rice Germplasm Center, 263 accessions of Oryza sativa were selected to represent diverse geographic origin, culture type, and each of six sub-specific groups. To ascertain if disease resistance is associated with sub-specific groups, the accessions were tested for resistance to 13 races of the blast (Bl) pathogen, Pyricularia oryzae; 6 races of the bacterial blight (BB) pathogen, Xanthomonas campestris pv. oryzae, and rice tungro virus (RTV). The temperate accessions of group VI (japonicas) were the most Bl susceptible. Tropical accessions of group VI (bulu and upland rices), and groups II, III, IV, and V were more Bl resistant than group I (indicas). Groups II, III, and IV were relatively resistant to BB, as were many deepwater rices. Group V was the most resistant to RTV. Sub-specific groupings may help target resistance screening efforts.

A335

THE EFFECT OF AGE, FLOWERING, AND SENESCENCE ON THE RESISTANCE OF TOBACCO TO BLUE MOLD. S. E. Wyatt and J. Kuć, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546

Age-related resistance to blue mold in tobacco is correlated with flowering. Exposure to short day conditions and cool temperatures (18C, 8 hr light) for 2-5 days ca. 1 month from seeding (f.s.) initiated premature flowering as early as 8 weeks f.s. At that time, plants were approximately 20cm in height, and had 10-12 fully expanded leaves. Control plants grown under long day conditions (8 hr light, 15 dark with a 1 hr night break) flowered in 12 weeks f.s. and were 120cm in height with 25-30 fully expanded leaves. These plants were resistant to blue mold caused by Peronospora tabacina, whereas tobacco induced to flower prematurely was highly susceptible. The prematurely flowering plants, however, became resistant as they aged. Resistance was correlated with the senescence of lower leaves. Thus, it would appear that the age of plants, not flowering, is the determining factor in age-related resistance to blue mold.

A336

FIELD ASSESSMENT OF THE GENETICS OF PHOMOPSIS RESISTANCE IN NARROW-LEAFED LUPINS. W.A. Cowling, Department of Agriculture, Baron-Hay Court, South Perth, Western Australia 6151.

Latent stem infections by Phomopsis leptostromiformis form lesions on narrow-leafed lupins (Lupinus angustifolius) during senescence. Resistance was measured in field experiments as a reduction in Phomopsis stem ratings in plots before seed harvest. Over 3 years of testing, parent lines and cultivars were classed as very resistant (VR), resistant (R), moderately resistant (MR), susceptible (S) and very susceptible (VS). Random F2-derived lines from crosses among parents belonging to these categories were tested for Phomopsis resistance in the F4 and F5. No susceptible progeny were identified in 243 bulks from 20 crosses among VR, R and MR parents, and only 15% of progeny were susceptible in 123 bulks from 13 crosses of VR or R with S or VS. More than one dominant gene for resistance may exist, one of which may be epistatic to another, but more MR and S progeny are predicted by these hypotheses than were identified in these experiments.

A337

THE VARIETY COLOMBIA, A COMPOSITE COFFEE CULTIVAR RESISTANT TO LEAF RUST (HEMILEIA VASTATRIX BERK. & BR.). J. Castillo, G. Moreno, G. Alvarado and G. Cadena. Centro Nacional de Investigaciones de Café (Cenicafé). Chinchiná, Colombia.

The coffee leaf rust was detected in the Western Hemisphere in 1970. The development of a composite cultivar was considered the best breeding strategy to use specific resistance safely and to confront a variable pathogen as Hemileia vastatrix, on a perennial crop such as coffee. After 20 years of research such cultivar was selected in Cenicafé, Colombia. The Caturra variety was used as the base cultivar and the Timor Hibryd as the donor parent, with more than five genes for specific resistance and other genes for partial resistance. About fifty progenies (F5) have been selected, that display high yield, complete resistance to the rust, phenotype uniformity and good bean and liquor quality. Since 1983 more than 200.000 hectares have been planted with a mixture of such progenies, which has been named Variety Colombia. Until now its resistance has remained stable.

A338

COMPARISON OF FIELD SCREENING METHODS TO EVALUATE RESISTANCE OF SOYBEAN CULTIVARS TO FROGEYE LEAF SPOT. C.N. Akem, K.E. Dashiell, and A.M. Emechebe. Grain Legume Improvement Program, International Institute of Tropical Agriculture, Ibadan, and Institute for Agricultural Research, Zaria, Nigeria.

Different methods were compared in field plots at two locations in Nigeria, to evaluate 4 soybean cultivars (cvs) for resistance to Cercospora sojina. Entries were arranged in a completely randomized block design with 4 replications. At both locations, planting infected seed of a susceptible soybean cv as a border row 2 wks before establishing the main plot, was most effective in obtaining early and maximum disease build up. This method resulted in a significantly (P=0.05) higher disease severity and disease incidence (DI) of frogeye leaf spot at the R1 growth stage. At the R7 stage, however, there was significant difference in disease among, but not within cvs, suggesting possible interplot interference. This test demonstrates the effectiveness of using infected border rows established early, to increase disease pressure needed in screening for disease resistance under field conditions.

A339

EXPRESSIONS OF RESISTANCE TO EXTRACELLULAR ENZYMES OF SNOW MOLD FUNGI IN ANTHER CULTURE DERIVED CALLUS SYSTEMS. F. Mehdizadegan and J. H. McBeath. Agricultural and Forestry Experiment Station, University of Alaska Fairbanks, Fairbanks, AK 99775.

Calli derived from anther culture of the winter wheat cultivar Roughrider were screened for expressions of resistance (or tolerance) to extracellular enzymes of snow mold fungi Sclerotinia borealis and sclerotial low temperature basidiomycete (sLTB). Over 2,000 calli with green centers (leaf primordia) from 16 clones were treated with snow mold enzymes (consisting mainly of cellulolytic and pecteolytic enzymes) and incubated at 10 C for 4 weeks. Calli were evaluated weekly and their extent of reactions was recorded using a numerical rating (0-4). The responses of calli to snow mold enzyme treatments varied widely, ranging from no change (rated 0) to complete discoloration and death (rated 4). Among all calli treated, 2.6% and 3.4% showed degrees of resistance to enzymes of S. borealis and sLTB, respectively.

A340

THE EFFECT OF FLUSILAZOLE ON THE GERMINATION OF CONIDIA OF FLUSILAZOLE-SENSITIVE AND -RESISTANT ISOLATES OF *VENTURIA INAEQUALIS*. Franzine D. Smith and Wolfram Köller, Cornell Univ., NYSAES, Geneva, 14456.

The effect of flusilazole on germtube elongation and appressorium formation by conidia of flusilazole-sensitive (S92) and -resistant (W10) isolates of *V. inaequalis* was studied. In this study, resistance to flusilazole was expressed at an early stage of conidial germination and the degree of resistance was transient. Also flusilazole acted as an appressorial inducer. Germtube elongation of W10 was not affected at 1 µg flusilazole/ml and not fully inhibited at 10 µg/ml by 16 h germination and through 38 h it was inhibited only 12% at 0.001µg/ml. Germtube elongation of S92 was completely arrested 8 h after germination at 0.001 µg/ml and 50% inhibition occured after 4 h germination at 1 µg/ml. The ED-50 value of W10 based on germtube elongation decreased over time. At 0 - 8 h the ED-50 value was >10 µg/ml and between 32 and 38 h the ED-50 value was 0.006 µg/ml. The greatest decrease in ED-50 occurred between 24 and 36 h. On water agar only 5 % of the untreated conidia formed appressoria by 44 h, but when conidia were treated with flusilazole, appressoria were differentiated between 24 and 32 h. Appressorium differentiation was dose dependent but did not exceed 80 %.

A341 Withdrawn

A342

SUSCEPTIBILITY OF CITRUS FRUIT TO XANTHOMONAS CAMPESTRIS PV. CITRUMELO AND PV. CITRI. J. H. Graham, T. R. Gottwald, T. D. Riley, and M. A. Bruce. University of Florida, CREC, Lake Alfred, FL 33850 and USDA-ARS, Orlando, FL 32803.

Valencia and Hamlin sweet orange, Ruby Red and Marsh grapefruit, and Orlando tangelo fruit were inoculated biweekly in the field at different stages of growth with an aggressive strain of X.c. citrumelo (F1). Various concentrations of F1 were applied as pressurized sprays (ps) to watersoak the rind tissue and were compared to a noninoculated ps and a gentle spray (gs) at 10^6 cfu/ml. The gs and ps at 10^4 cfu/ml yielded erratic infection within the watersoaked zone whereas the ps 10^8 and 10^6 consistently produced lesions within and outside the treated area. Disease rating decreased as fruit size increased more rapidly at ps 10^6 than at ps 10^8. The period of susceptibility was greater for grapefruit than for orange, with tangelo intermediate. Lesions did not expand in size after 2 wk and bacteria did not multiply in lesions, i.e. citrus fruit were not susceptible to X.c. citrumelo. A similar relationship between fruit size and susceptibility was shown for X.c. citri in Argentina.

A343

A SEMISELECTIVE MEDIUM FOR THE DETECTION AND ISOLATION OF XANTHOMONAS CAMPESTRIS PV. VESICATORIA FROM TOMATO SEEDS. K. Sijam[1], C.J. Chang[2], and R.D. Gitaitis[3]. [1]Department of Plant Protection, Universiti Pertanian Malaysia, 43400 Serdang, Selangor, Malaysia, and Department of Plant Pathology, University of Georgia, [2]Georgia Station, Griffin, GA 30223, and [3]Coastal Plain Station, Tifton, GA. 31793.

A semiselective medium was developed for the isolation and preliminary identification of Xanthomonas campestris pv. vesicatoria from tomato seeds. Selectivity was afforded through the use of cycloheximide, bacitracin, neomycin, cephalexin, 5-fluorouracil and tobramycin, while Tween 80 was included for colony differentiation. All X.c. pv. vesicatoria isolates can be distinguished from other contaminating

bacteria in the seeds by the formation of clear rings around their colonies. X.c. pv. vesicatoria was found in 13 of 23 seed lots which previously tested negative on other selective media. Recovery of up to 100% was achieved compared to Tween B medium.

A344

COLONY HYBRIDIZATION WITH TI PLASMID PROBES TO *AGROBACTERIUM* ISOLATES FROM APPLE ROOTSTOCK TUMORS. M. L. Canfield, M. D. Kawalek and L. W. Moore. Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331-2902

Crown gall in apple rootstocks has been difficult to study due to the inability to isolate tumorigenic strains from galls. A new method for identifying putative *A. tumefaciens* strains was therefore attempted. Isolations were made from tumors on Emla 7, Mark and Domestic Seedling rootstocks from two nursery sites in Washington state. Nineteen tumors were sampled and dilution plated onto Kerr 1A, Kerr 2E and Roy and Sasser semiselective media for biovar strains 1, 2 and 3 respectively and onto a rich mannitol-glutamate medium supplemented with several salts, trace elements and yeast extract. Two hundred and fifty isolates were collected from each tumor and tested for the presence of Ti plasmid DNA using ^{32}P labled probes. Colony hybridizations were carried out using a 7.5 kb fragment of pTiB6806 including tms 1 and tmr loci and a 25.2 kb fragment of PTiA6 spanning the virA, virB, virF and virG loci. Isolates of one tumor from Domestic Seedling rootstock showed hybridization to both probes in 79% of the colonies tested, while isolates of other tumors from Mark rootstock had no colonies which hybridized to either probe.

A346

PLASMID TRANSFER BETWEEN AGROBACTERIUM RADIOBACTER K84 AND A. TUMEFACIENS IN CROWN GALL TISSUE. V. O. Stockwell[1], M. D. Kawalek[1], L. W. Moore[1], and J. E. Loper[2], [1]Dept. Botany and Plant Path., Oregon State University, and [2]USDA-ARS, HCRL, Corvallis, OR 97331.

Agrobacterium radiobacter strain K84 affects biocontrol of crown gall disease due, in part, to plasmid-conferred production of agrocin 84. Plasmid transfer between A. radiobacter strain K84 and an agrocin 84-resistant pathogen, A. tumefaciens strain B49c, was evaluated in galls of four plant hosts maintained in a growth chamber. Transfer of pTiB49c::Tn5, the tumor-inducing plasmid of strain B49c, to K84 was not detected in sunflower, Kalanchoe, or cherry but was detected in one of 25 tomato galls. Transfer of pAgK84::Tn5, the plasmid conferring agrocin 84 immunity and production, from strain K84 to strain B49c was detected commonly in tomato and sunflower galls at a mean frequency of 10^{-3} per recipient. B49c(pAgK84::Tn5) transconjugants were detected in none or one of 20 galls of Kalanchoe or cherry, respectively. In all cases, plasmid exchange was detected only when populations of K84 and B49c exceeded 10^5 cfu/g gall tissue, and was not related to the presence of opines. In field trials, transfer of pAgK84 to strain B49c was detected in 5 of 11 galls of coinoculated cherry seedlings that supported at least 10^5 cfu of K84 and B49c per gram of gall tissue. Results suggest that Agrobacterium strains harboring both pTi and pAgK84 may arise from plasmid transfer in crown gall tissue in the field.

A347

DISTRIBUTION AND SEVERITY OF BACTERIAL BLIGHT OF RICE IN NEPAL. T. B. Adhikari, T. W. Mew and Paul S. Teng, International Rice Research Institute, P.O. Box 933 Manila, The Philippines.

Systematic surveys for bacterial blight (BB) of rice, caused by Xanthomonas campestris pv. campestris were conducted in 32 major rice growing districts of Nepal during 1987-1989. A total of 170 rice fields was examined and disease severity (percent of leaves blighted/plant) was recorded on 1 m^2 area in each field. BB was found to be prevalent throughout the rice growing areas of the country. Severity was highest (>50%) in Jhapa, Morang, Bara, Parsa and Kanchanpur of Tarai districts and lowest in Gorkha and Nuwakot of the Hilly districts. Average disease severity ranged from 30% - 40% in tarai and 15% - 20% in hills and valleys. Differential reactions were evident across the sites for some rice cultivars. Masuli, susceptible to BB was extensively grown in 23 surveyed sites. Likewise, CH-45, Himali, Sarju-49 and Bindeshwori were found highly susceptible to the disease. Results suggest that BB could be a major rice disease of epidemic proportions in Nepal.

A348

TRANSMISSION FREQUENCIES OF DIFFERENT ISOLATES OF CITRUS GREENING USING DIFFERENT METHODS OF GRAFT INOCULATION. R. F. Lee[1], R. H. Brlansky[1], M. E. Hooker[2], E. L. Civerolo[2], and H. Garnett[3], [1]University of Florida, Lake Alfred 33850, [2]USDA-ARS, Beltsville, MD 20705, and [3]University of Wollengong, NSW, Australia.

Graft transmission of citrus greening was attempted by using leaf pieces, blind buds, side shoots, or approach grafts. Four different isolates were used and the tests were conducted on Citrus jambhiri Lush. and C. paradisi Macf. Positive transmission was determined by visual symptoms and the presence of gentisic acid. Each greening isolate produced its own characteristic symptoms, but all symptoms were manifested better in C. paradisi than in C. jambhiri. Transmission was best achieved by side shoot and approach grafting and was the least with leaf pieces.

A349

Characterization of Xanthomonads from Aroids. **A. R. Chase**, University of Florida, IFAS, Central Florida Research and Education Center-Apopka, FL 32703.

Approximately 150 strains of *Xanthomonas campestris* pv. *syngonii* and *X. c. dieffenbachiae*, obtained from ornamental and agronomic Aroid plants were characterized by pathogenic and physiologic reactions. These strains were from plants in the following genera: *Aglaonema*, *Anthurium*, *Colocasia*, *Dieffenbachia*, *Epipremnum*, *Philodendron*, *Syngonium*, and *Xanthosoma*. Pathogenicity tests with *Aglaonema*, *Anthurium*, *Dieffenbachia*, *Philodendron* and *Syngonium*, groups of strains were more virulent on their host of origin, but were not host specific. Strains from some host genera showed different characteristics based on minimum pH for growth, copper and streptomycin resistance, pectolytic activity, and starch utilization on four media, although no single characteristic or combination of characteristics could be used to separate one group of strains from the rest. The degree of differences in pathogenic and physiologic reactions indicate the heterogeneous nature of *X. c.* pv. *dieffenbachiae* but does not support separation into more than one pathovar, at this time.

A350

EFFECT OF SUGARCANE CULTIVAR SUSCEPTIBILITY ON SPREAD OF RATOON STUNTING DISEASE BY THE MECHANICAL HARVESTER. K. Damann, Dept. of Plant Pathology, LAES, LSU Agricultural Center, Baton Rouge, LA 70803.

Disease-free sugarcane cultivars susceptible (L 62-96), intermediate (CP 65-357), and resistant (L 60-25) to ratoon stunting disease (RSD) were planted in the fall of 1986 and harvested in the fall of 1987. Inoculum was provided by diseased plants preceding each row. Stalk samples were collected every 0.3m from the first 12.2m of each row in first ratoon and assayed for RSD in the fall of 1988. Sampling was repeated in second ratoon in the fall of 1989 and incidence determined. RSD incidence in L 62-96 preceded by 1, 2, or 4 diseased plants was 30, 60, and 75% re-spectively, in 1988 (after 1 harvest) and increased to 84, 98, and 99% in 1989 (after 2 harvests). Incidence in CP 65-357 was 10, 11, and 9% in 1988, and increased to 25, 24, and 44% in 1989. No spread was detected in L 60-25. Gradients were apparent when incidence in consecutive 3.05m row segments was determined. Little spread occurred beyond the first segment in CP 65-357. Fourth segment incidence in L 62-96 preceded by 1, 2, or 4 diseased plants averaged 4, 38, and 43% respectively.

A351

A METHOD FOR THE DETECTION OF SEEDBORNE BACTERIAL PATHOGENS IN TOMATO...D.A. Maddox and J.P. Hubbard Asgrow Seed Co. San Juan Bautista, CA 95045

Residual chlorine (cl) was detected when calcium hypochlorite (CaOCl) treated tomato seedlots were assayed, using the Stomacher blender and liquid plating technique. Cl levels greater than 20 ppm interfered with the recovery of bacterial pathogens in the assay. To eliminate the effects of residual Cl, 0.2% sodium thiosulfate (STS) was added to the 0.05M phosphate extraction buffer (pH 7.2). STS amended buffer reduced residual Cl of treated seedlots to undetectable levels. When Clavibacter michiganense subsp. michiganense Xanthomonas campestris pv. vesicatoria and Pseudomonas syringe pv. tomato were added to treated seedlots and assayed with STS amended buffer, all three bacteria were recovered at expected levels. The recovery of all three pathogens was better with STS amended buffer than with unamended buffer, when artifically infested seeds were added to CaOCl treated seedlots. Assays of CaOCl treated tomato seedlots with STS amended buffer may help in determining the efficacy of CaOCl to control seedborne bacterial pathogens.

A352

PECTOLYTIC CLOSTRIDIA ASSOCIATED WITH SOURING OF SWEETPOTATO. V. Duarte, and C. A. Clark. Dept. Plant Pathology & Crop Physiology, Louisiana Agric. Expt. Sta., LSU Agric. Center, Baton Rouge 70803-1720.

Storage roots of cv. Beauregard were either not treated (control), disinfested with 1% NaOCl for 10 min, or inoculated with *Erwinia chrysanthemi* (Ech-2); and half of the roots from each treatment were punctured with a flamed needle. They were then incubated submerged in distilled water in plastic bags for 5 days at 25 C. Souring symptoms were present in 93 and 8, 8 and 0, and 100 and 60% of punctured and non-punctured roots from the control, disinfested, and Ech-2-inoculated treatments, respectively. *E. chrysanthemi* was isolated only from artificially inoculated storage roots. However, at least two pectolytic spore-forming, sulfate-reduction negative, gelatin hydrolysis positive, anaerobic bacteria resembling *Clostridium* spp. were found in most lesions, regardless of treatment. Isolates of the two clostridia macerated sweetpotato, potato, carrot, and onion, but symptoms were distinct from the sticky rot symptom reported previously. Among other characteristics, the two clostridia can be differentiated by the diameter (1 and 2.5 mm) of pitting on Lund's double-layer pectate medium after 2 days at 32 C. Further characterization of the clostridia is in progress.

A353

THE EFFECT OF AMENDMENT ON THE POPULATION OF PSEUDOMONAS SOLANACEARUM AND THE INCIDENCE OF BACTERIAL WILT OF TOMATO. G. L. Hartman and C. H. Yang, Asian Vegetable Research and Development Center, P.O. Box 42, Shanhua, Tainan 74199, Taiwan, R.O.C.

The population of Pseudomonas solanacearum in soil was reduced from 2.5×10^5 cfu/g to 5.6×10^2 cfu/g of dry soil after 40 days when mixed by weight with 1% amendment (98% mineral ash and 2% urea) under laboratory conditions. Between 18 and 21 days after the addition of the 1% amendment, the bacterial population was reduced to <50% of the initial population. In the greenhouse, a highly susceptible tomato line (L 390) was transplanted to a 1% amended soil in pots. Eighty-percent of the plants survived in amended soil versus complete mortality of plants in soil without amendment. In field plots, the population of P. solanacearum was significantly less after 54 days in amended plots than in plots without amendment. The survival rate of L 390 at 47 days after transplanting in the field was 65% in the amended plots and 20% in plots without amendment.

A354

A REFINED MASS SCREENING TECHNIQUE FOR RESISTANCE TO PSEUDOMONAS SOLANACEARUM. El-Nashaar, Hossien M., De Lindo, L. and Nydegger, U. International Potato Center (CIP), Apartado Postal 5969, Lima 100, Peru.

A technique for mass screening of potato genotypes to select for resistance to Pseudomonas solanacearum was developed. Plants grown from cuttings, microtubers or true potato seeds were transplanted into Jiffy-7 peat pellets, and grown until a root system was developed. Established roots were submerged in an aqueous inoculum suspension for 10 seconds. Plants were evaluated after 4-days of incubation at 27-32 C and every 2-days thereafter for up to 14 days. Concentration of inoculum ($5X10^5$ - $1X10^8$), reproduction methods, and physiological age of cuttings critically influenced symptom development. When roots were wounded none of the tested genotypes survived beyond 15 days after inoculation. Unwounded plants survived beyond 35 days. Advantages of this technique are: 1) no physical wounding is required; 2) variability due to root to root contact is minimized; 3) 40 or more plants can be inoculated in 10 seconds.

A355

IMPORTANCE OF WOUNDS AS INFECTION COURTS FOR POSTHARVEST DECAY OF PEAR BY PHIALOPHORA MALORUM. David Sugar and R.A. Spotts, Oregon State University, Medford 97502.

Bosc pears were not infected by Phialophora malorum via lenticels nor intact interlenticellular surface areas following 6 mo exposure to various inoculum concentrations at 0°C. Punctured lenticels (0.75 mm wound diameter) were consistently infected. Bruising of lenticels by impact of a 142 g steel bolt dropped from 5-20 cm prior to inoculation did not result in infection, regardless of immersion depth in inoculum suspension. Of 185 non-bruised lenticels dissected following soaking in methylene blue solution, only 4 were penetrated by dye solution. Tissue beneath lenticels was penetrated by dye solution following impact from 15 and 20 cm. Germination of conidia of P. malorum was enhanced in water in which wounded or wax-removed but not intact pears had been soaked.

A356

ANTHRACNOSE OF CITRUS FRUIT CAUSED BY COLLETOTRICHUM GLOEOSPORIOIDES IS ENHANCED BY POSTHARVEST HANDLING. G. Eldon Brown, Florida Department of Citrus, Citrus Research and Education Center, Lake Alfred, FL 33850

Anthracnose develops postharvest mostly on early season Florida citrus fruit which have to be ethylene-degreened. The disease is most prevalent on the Robinson tangerine cultivar, particularly on those fruit with the poorest color following degreening. Last season, significant commercial losses from anthracnose also occurred on grapefruit. To assess the effect of handling procedures on anthracnose, Dancy tangerines were subjected to different treatments of degreening, washing and waxing. Higher than standard degreening temperatures (35 vs. 30C) delayed anthracnose development. Disease incidence and severity were increased by higher than recommended ethylene concentrations (50 vs. 5 ppm), washing, and by waxing with resin solution water wax rather than with solvent wax or no wax. The greatest stimulation occurred on degreened (50 ppm) and washed fruit, on which the surface area affected by anthracnose was increased from 1.5 to 44.9% by applying water wax.

A357

ISOLATION, PURIFICATION AND BIOLOGICAL ACTIVITY OF A NEW TOXIN (HM-8) FROM Fusarium culmorum HM-8. Hamed K. Abbas and C. J. Mirocha, Dept. of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

An isolate of F. culmorum (W.G. Smith), Sacc. HM-8 from a scabby wheat kernel sample from England produced a toxin in culture when grown on rice grains in the laboratory. This new toxin, given the trivial name of HM-8, caused food refusal, weight loss in rats, cytotoxic effects to mouse and human fibroblast cells at concentrations of 2.5 μg/ml and 7.5 μg/ml, respectively, and mortality to chick embryos (10 to 70%) at a concentration range of 0.5 μg to 4 μg per egg. HM-8 showed UV absorption maxima at 210 and 285 nm and does not fluoresce under UV light. It exists as powdery white crystals with a melting point range from 181 to 182°C. HM-8 showed bright blue fluorescence after its reaction with 20% sulfuric acid in MeOH or acidic p-anisaldehyde. Elemental and accurate mass determinations in both electron impact (EI) and chemical ionization (CI) indicate an empirical formula of $C_{11}H_{14}O_5$. HM-8 is a carbohydrate with a hemiacetyl group. This is the first report of this compound, HM-8, produced by F. culmorum.

A358

ASPERGILLUS FLAVUS PHENOTYPES VARYING IN REGULATION OF AFLATOXIN BIOSYNTHESIS. E.H. Gendloff, F.S. Chu, and T.J. Leonard. University of Wisconsin, 430 Lincoln Drive, Madison, WI 53706.

We are interested in identifying and characterizing genetic variations in regulation of aflatoxin biosynthesis among wild-type Aspergillus isolates. Aflatoxin production by previously described isolates has been shown to be very low or lacking when grown in a peptone/minimal salts medium without sugar (PMS), and delayed more than two days in a maltose/minimal salts medium (MMS). We have identified two wild-type isolates of Aspergillus flavus, NRRL 6554 and SRRC 26, which make significant amounts of aflatoxin on PMS, as well as within two days of growth on MMS. We have also used mutagenesis on several wild-type aflatoxin non-producers or low producers to determine if any of these strains can be induced to produce large amounts of aflatoxin. Of seven strains tested, one of the low producers was induced by mutation to produce large amounts of aflatoxin. We are using parasexual analysis, with protoplast fusion when necessary, to determine dominance and allelic relationships among the phenotypes described above. Auxotroph isolation, required for this analysis, has been facilitated by selecting for mutants in nitrogen metabolism by the use of chlorate-containing growth medium.

A360

COMPARISON OF HARD WHITE AND HARD RED WINTER WHEATS IN STORAGE. D. M. Trigo-Stockli, J. R. Pedersen, and D. B. Sauer, Food and Feed Grains Institute, Kansas State Univ., and USDA-ARS, U. S. Grain Marketing Research Laboratory, Manhattan, KS 66506

Research on the production and utilization of hard white winter wheats has increased, but little is known about their susceptibility to storage fungi. Samples of hard white and hard red winter wheat were stored in plastic bags at 14, 16, and 18% moisture content and 25 C. They were tested over a 16-week storage period for moisture content, germination, and mold invasion. When stored at moisture contents of about 16 and 18%, the hard red winter wheat had lower percentages of surface disinfected kernels yielding Aspergillus glaucus compared to hard white winter wheats. Reduction in germination percentage was generally similar in the two wheat types, but at 16% moisture the hard red wheat decreased more than the hard white wheats.

A362

THE ROLE OF HYDROXY RADICAL IN THE HYPERSENSITIVE REACTION OF *CUCUMIS SATIVA* L. (CUCUMBER) TO *PSEUDOMONAS SYRINGAE* PV. *PISI*. P. L. Popham and A. Novacky, 108 Waters Hall, Dept. of Plant Pathology, University of Missouri, Columbia, Mo. 65211.

Active oxygen is involved in the hypersensitive reaction (HR) of plants to incompatible pathogens. The presence of lipid peroxidation correlates chronologically with the production of superoxide ($O_2^{\cdot-}$). However, $O_2^{\cdot-}$ may not be the active oxygen species involved. Evidence from other systems suggests that $O_2^{\cdot-}$ is converted to the hydroxy radical ($OH^{\cdot}$) before lipid peroxidation is initiated. Until recently $OH^{\cdot}$ could not be detected *in planta*; however, a colorimetric assay that utilizes the ability of $OH^{\cdot}$ to oxidize dimethyl sulfoxide forming methane sulfinic acid has recently been reported. Electrolyte leakage and lipid peroxidation occur during HR. This paper addresses the role of $OH^{\cdot}$ in HR and provides insight into the relationship of $OH^{\cdot}$ with electrolyte leakage and lipid peroxidation.

(Supported by NSF)

A363

TRANSGENIC TOBACCO PLANTS WITH INDUCED RESISTANCE TO FUNGAL ATTACK. K. Broglie, R. Broglie, M. Holliday, and I. Chet. Agricultural Products Dept., E.I. DuPont de Nemours and Co. (Inc.), Wilmington, DE. 19880

Chitin is an important component of most fungal cell walls. One method by which plants protect themselves against pathogens is by the production of lytic enzymes, such as chitinase, which enable them to partially hydrolyze these cell walls. In healthy, uninfected plants, chitinase levels are low or undetectable. However, treatment with ethylene, oligosaccharide elicitors, or infection with fungal pathogens results in an increase in chitinase mRNA levels and an increase in enzyme activity. Analysis of chitinase gene expression in transgenic tobacco have identified two promoter regions responsible for optimal gene expression. Transgenic tobacco plants containing a chimeric gene composed of a bean chitinase promoter fused to the coding region of the reporter gene β-glucuronidase (GUS) have been used to study chitinase gene expression in response to fungal infection. Our results indicate that gene activation occurs locally in tissues immediately surrounding the site of fungal infection and coincides with the induction of endogenous tobacco defense genes. The role of chitinase in plant protection has also been studied using transgenic tobacco plants which express a bean chitinase gene modified so that the native inducible promoter is replaced by a high level, constitutive promoter. These transgenic plants exhibit increased resistance to the pathogenic fungus *Rhizoctonia solani* resulting in less root damage and increased ability to survive in infested soil; however no effect of the non-chitinous fungus *Pythium* was found.

A364

STOMATAL AND MESOPHYLL LIMITATION OF PHOTOSYNTHESIS IN RESISTANT AND SUSCEPTIBLE ALFALFA INFECTED BY VERTICILLIUM ALBO-ATRUM. B.W. Pennypacker, D.P. Knievel, K.T. Leath, E.J. Pell and R.R. Hill, Jr. Penn State Univ. and USDA-ARS, U.S. Regional Pasture Research Lab., Univ. Park, PA 16802.

The effect of V. albo-atrum on net photosynthesis (P_n), stomatal conductance, stomatal limitation of P_n and activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) was determined in two greenhouse-grown alfalfa clones. A/Ci response curve analysis and leaf protein extraction and assimilation of $^{14}CO_2$ were used to determine the in vivo and in vitro activity of Rubisco, respectively. Rubisco activity, P_n, and stomatal conductance were reduced in the infected susceptible clone but stomatal limitation of P_n was not affected. Reduced P_n was attributed to reduction in carboxylating capacity. The pathogen had no effect on P_n, stomatal limitation of P_n or Rubisco activity in the resistant clone. Activity assays confirmed the in vivo finding that Rubisco activity was significantly reduced in the infected susceptible clone but not in the resistant clone.

A365

EFFECTS OF LIGHT ON THE GERMINATION OF SPORANGIOSPORES OF PERONOSPORA TABACINA AND DEVELOPMENT OF BLUE MOLD IN TOBACCO LEAVES. M. Peng and J. A. Kuć, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Experiments were conducted to determine whether a period of darkness (18-24 h at 18-22 C) was necessary for development of Peronospora tabacina Adam in tobacco leaves. Light (blue fluorescent, 86.5 $\mu Em^{-2}\ s^{-1}$) inhibited the germination of sporangiospores 50-75% in vitro and 40-60% on leaf discs. Sporulation and disease development of blue mold were markedly inhibited when plants (Nicotiana tabacum L. cv. Ky-14) were directly exposed to light for 18 h after inoculation. Temperature and humidity were unchanged for plants exposed to light or not. Exposure of two expanded leaves to light reduced blue mold development and sporulation not only on the surfaces exposed to light but also on surfaces kept shaded. This suggests a factor is transported from leaves exposed to light which is either inhibitory to P. tabacina or elicits resistance in leaves kept in the dark.

A366

MOLECULAR BASIS THAT RULES THE HOST RANGES OF PATHOTYPES OF ALTERNARIA ALTERNATA. K. Kohmoto, Y. Itoh, M. Kodama, H. Otani, and S. Nakatsuka*, Plant Pathology Lab., Fac. of Agric., Tottori University, Tottori 680, Japan and *Organic Chemistry Lab., Fac. of Agric., Nagoya University, Nagoya 464, Japan.

The collective species of A. alternata contains many pathotypes affecting certain genotypes of diverse plants. Of them, Japanese pear, strawberry and tangerine pathotypes produces highly potent host-specific toxins(HST) which share ω-epoxy-8-hydroxy-9-methyldecatrienoic acid. Although the structures are very similar each other, their toxicity is definitely selective. AK-toxin from Japanese pear pathotype is toxic to only susceptible pear. AF-toxin I from strawberry pathotype is toxic to certain pear as well as susceptible genotype of strawberry. ACT-toxin I from tangerine pathotype is toxic to tangerines and mandarins susceptible in the field, and toxin II, the 5"-deoxy derivative of toxin I, is harmful to certain pear that are experimentally found host. The sensitive plant range to each HST is coincident with the host range of each pathotype. Release HST on germination can induce the accessibility to penetration at infection sites in susceptible genotypes.

A367

REVERSIBLE INHIBITORY EFFECTS *IN VITRO* AND *IN VIVO* OF A RESISTANCE COMPOUND FROM MAIZE. Frank A. Cantone and Larry D. Dunkle, USDA-ARS, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907

Resistance of maize to *Helminthosporium carbonum* race 1 is induced by prior inoculation with race 2, a nonpathogen. Appressorium formation, penetration, and hyphal growth by the pathogen, as well as development of large lesions typical of the susceptible reaction are decreased in induced tissue. The induced resistance is consistently associated with the presence of a compound(s) in diffusates on the leaf surface which inhibits conidial germination and germ tube elongation, prevents incorporation of labeled precursors into protein and RNA, and substantially reduces respiration. The inhibitory effects are reversed when conidia are washed with water or when organic or amino acids are added to the conidial suspension. The addition of sodium acetate to race 2 or challenge race 1 inoculum on the leaf negates the inhibitory activity of the diffusates, abolishes induced resistance, and results in formation of susceptible lesions. Resistance as well as production of the inhibitory compound(s) is induced by other fungi and in other maize lines inoculated with *H. carbonum*. The results suggest that a general resistance mechanism is activated upon contact of the maize leaf with a potential pathogen.

A369

ISOLATION OF GENES ENCODING ROOT-KNOT NEMATODE STYLET EXUDATE PROTEINS. I. Kaloshian and D. McK. Bird. Department of Nematology, University of California, Riverside, CA 92521.

DNA sequence has been inferred from a 32 amino acid stretch of a 212 k glycoprotein component of the *M. incognita* stylet exudate, and an oligonucleotide (23-mer; 1,024 X degenerate) synthesized. Southern blots of *M. incognita* DNA were probed with kinased oligomer and washed at 53°C in 3M TMAC; two *Eco* RI fragments were detected (7 kb and 3.5 kb). A 3.5 kb fragment also was observed in *M. javanica*. Screening with cDNA, Taylor-primed from *M. incognita* total RNA, indicated that the 3.5 kb, but not the 7 kb, fragment lies within the ribosomal repeat. The 7 kb *Eco* RI fragment has been cloned, and found to contain two closely spaced regions of homology with the oligomer. Different *Meloidogyne* spp. vary in their dosage of this sequence. On-going analysis will reveal if this recombinant encodes the 212 k protein.

A370

PRODUCTION OF TOXINS WITH EQUIVALENT HOST SPECIFIC ACTIVITY, FUMONISIN AND AAL, BY NONPATHOGENS OF TOMATO. C.J. Mirocha, D.G. Gilchrist, Ann Martensen, H.K. Abbas, J. Plasencia, and R.F. Vesonder, Departments of Plant Pathology, University of Minnesota and California (Davis); and USDA/ARS, NRRC, Peoria, IL.

Alternaria alternata f. sp. lycopersici produces a host specific toxin (AAL) differentially toxic to alleles of the asc gene in tomato. Fusarium moniliforme produces a closely related chemical derivative of AAL called FUMONISIN which causes identical necrotic lesions on the same tomato genotypes as AAL. In rat hepatoma tissue culture, AAL was inactive (ED of 50,000 ng/ml) whereas FUMONISIN was active (ED 5,000 ng/ml). A. alternata and F. moniliforme were grown on a rice substrate in the laboratory and fed (ad libitum) to rats in a toxicity study. Both cultures killed rats in less than 24 hrs. Stomach intubation of pure AAL (3.5 mg rat) and FUMONISIN (21 mg/rat) did not elicit toxic signs in rats. The toxicity of the fungus cultures was due to toxins other than AAL and FUMONISIN. These results challenge coevolutionary linkage of host specific toxins and pathogenicity and consider the biological basis for synthesis of these complex molecules.

A372

CORN STALK SENESCENCE VERSUS CORN STALK ROT. J. E. Partridge, B. L. Doupnik, and D. S. Wysong. University of Nebraska, Lincoln, NE 68583-0722.

Corn hybrids were selected to provide a range of maturities from short to full season. Fourteen hybrids were evaluated in each of four tests over a span of 10 years. Crushability, determined by hand squeezing, second internode above the brace roots, was used as the criterion for evaluation. Data collection was begun prior to any loss of green color in the stalks, and continued weekly until three weeks past killing frost. Conclusions drawn include: 1) there is little or no host resistance to Fusarium spp., 2) the rate curve for stalk senescence is hybrid dependent and predictable, 3) stalk senescence is often reported as stalk rot, and 4) stalk lodging vulnerability can be predicted and managed to reduce losses due to "stalk rot".

A373

FIELD STUDIES ON SUDDEN DEATH SYNDROME OF SOYBEAN. S. B. Belmar and H. W. Kirby, Department of Plant Pathology, University of Illinois, Urbana, IL 61801.

A three year field study was conducted in Illinois to evaluate soil applied pesticides for the control of sudden death syndrome (SDS) of soybean. Plots treated with methyl bromide lacked disease symptoms, i.e. interveinal chlorosis and necrosis of the leaflets. Application of Ridomil 5G, Terraclor 10G, and Imazalil 5G did not consistently reduce foliar symptom development compared to a non-treated control. Populations of soybean cyst nematode were reduced with Temik 15G; however, control of SDS was not observed. Disease symptoms were more severe with an "early" planting than with a "late" planting of soybeans. Regardless of planting date, symptoms consistently appeared in Illinois when plants were in the early pod growth stage. Seed harvested from plants with severe SDS symptoms were planted in methyl bromide treated soil; however, no SDS foliar symptoms were observed. Therefore, the causal organism did not appear to be transmitted by seed in this study.

A374

OBSERVED SPREAD OF SOYBEAN STEM CANKER DISEASE. B. L. Keeling, USDA-ARS, Jamie Whitten Delta States Research Center, Stoneville, MS 38776

The spread of soybean (Glycine max) stem canker disease, caused by Diaporthe phaseolorum Var. caulivora, in a susceptible cultivar (J77-339) from a point source of inoculum was measured. Data was taken from field plots during two growing seasons. During the first season, plots were subjected to rain and wind from the remnants of two hurricanes. The disease was spread in only one direction to a maximum of 6.4 m. Spread of the disease reflected the influence of the weather pattern. During the second year, plots were subjected to several periods of rain without strong winds. Diseased plants occurred randomly dispersed in all directions from the source of inoculum. In both years, most diseased plants occurred within 2 m of the source of inoculum.

A375

RHIZOPUS ROOT ROT AND PHYTOPHTHORA ROOT ROT OF SUGAR BEET IN WYOMING. P. C. Vincelli, C. M-S. Beaupré, and W. F. Wilcox*, Dept. of Plant, Soil & Insect Sciences, Univ. of Wyoming, Laramie, 82071 and *Dept. of Plant Pathology, New York State Agric. Exper. Stn., Geneva, 14456.

Two root diseases previously unreported from Wyoming were identified in sugar beets from commercial production fields in Washakie County during 1988-89. Rhizopus root rot, caused by *Rhizopus arrhizus*, was observed in a field in 1988. The disease was most severe in a portion of the field with poor surface drainage, where over 50% of the plants were affected. Poor drainage, temperatures well above normal, and insect injury were thought to have promoted development of the disease. Phytophthora root rot was diagnosed in sugar beet tap roots submitted to the UW Plant Disease Clinic following the 1989 harvest. Although *Phytophthora drechsleri* has been reported as causing root rot of sugar beet in other states, the isolate obtained in Wyoming more closely fit the description of *Phytophthora cryptogea* based on morphology of sporangia and sporangiophores and temperature growth relations. Excessive soil moisture, possibly caused by overirrigation, was thought to have promoted disease development. Although *Rhizoctonia solani* is the most common root rot pathogen of sugar beet in Wyoming, these previously unreported pathogens may occasionally be responsible for root rot, as well.

A376

POPULATION DYNAMICS OF *FUSARIUM OXYSPORUM* AND *PYTHIUM* SPP. WITHIN ASYMPTOMATIC PLANTS IN A SOUTH DAKOTA SOYBEAN FIELD. C. M-S. Beaupré, M. W. Ferguson*, and G. W. Buchenau**, Dept. of Plant, Soil & Insect Sciences, Univ. of Wyoming, Laramie,

82071-3354, *Biology Dept., Coastal Carolina College, Conway, SC 29526-1954, and **Plant Sciences Dept., South Dakota State Univ., Brookings, SD 57007-2207.

Fusarium oxysporum and *Pythium* spp. populations within soybean plants, and the effects of benomyl and metalaxyl upon these, were monitored over 1984, 1985, and 1986 in a field having a history of moderate Fusarium root rot of soybean. Endophytic populations of both *F. oxysporum* and *P. ultimum* were present in asymptomatic plants. *Pythium ultimum, P. irregulare, P. dissotochum, P. acanthicum,* and two *Pythium* spp., 'group HS' were most numerous during early seedling development; gradually being replaced by *F. oxysporum*. Endophytic population levels of *F. oxysporum* and *Pythium* spp. had no apparent effect upon each other, but appear to be more dependent upon host plant development and/or environment. Benomyl seed and foliar treatment and metalaxyl side dressing at planting reduced endophyte populations, but did not eliminate these fungi.

A379

A RESISTANCE-GENE-BASED NAMING SYSTEM FOR RACES OF PLASMOPARA HALSTEDII. W.E. Sackston, Macdonald College of McGill University, Ste. Anne de Bellevue, Que., Canada, H9X 1C0, T.J. Gulya, and J.F. Miller, USDA-ARS, Northern Crop Sciences Laboratory, Fargo, ND 58105

Resistance in sunflowers to downy mildew (Plasmopara halstedii) prior to 1980 was conferred by two genes, Pl 1 and Pl 2 and their equivalents, Pl 3 and Pl 4 respectively, and two races of the pathogen had been identified. By 1989 eight genes for resistance had been identified or postulated. According to the gene-for-gene hypothesis, if all eight prove to be distinct and are independently inherited, they could differentiate up to 2^8 or 256 races. Numerical designation of races based on the sequence of their discovery or description provides little or no information about their pathogenic potential to plant breeders, epidemiologists, or other specialists. To make designations of sunflower downy mildew races informative and useful everywhere in the world, we propose that they be based on the resistance genes which the respective races can overcome.

A380

EFFECT OF MOISTURE ON SEPTORIA NODORUM BLOTCH OF WHEAT. S.E. PENIX and E.A. Milus, Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Under field conditions in Arkansas, septoria nodorum blotch is favored by two or more days/wk of measurable rainfall. Laboratory and greenhouse studies determined the effect of free moisture on lesion development on moderately resistant (Florida 302) and susceptible (Caldwell) cultivars. On Caldwell seedlings, the percent leaf area diseased increased as the post-inoculation dew period increased from 12-30 hr (6 hr increments, 12, 17, 22C). However, on Florida 302 seedlings, the percent leaf area diseased remained low for all dew periods. In the greenhouse studies, lesions enlarged rapidly on vernalized Caldwell plants at the tillering stage as dew period increased from 24-72 hr (12 hr increments, at ambient air temperature), but lesions enlarged slowly on Florida 302 for all dew periods. At the flowering stage, the rate of lesion enlargement with increasing dew period was similar on both cultivars.

A381

THE INCIDENCE OF FUSARIUM SPECIES IN STEM BASES OF WINTER WHEAT IN THE MIDLANDS, U.K. D.W. Parry, Harper Adams Agricultural College, Newport, Shropshire, TF10 8NB, U.K.

In a survey of a total of nine winter wheat crops in the years 1987, 1988 and 1989, the predominant Fusarium species isolated from stem bases was Fusarium nivale. Fusarium avenaceum, F. culmorum and F. graminearum were also isolated. The highest incidence of F. nivale occurred during April, 1989 in the cultivar Brock when the fungus was isolated from 65% of the tillers sampled. The highest incidence of F. avenaceum was 60% (August 1988, cv. Slejpner) and F. culmorum 37% (August, 1989, cv. Mercia). A delay in onset of infection during 1987 was attributed to the low January temperatures and an upsurge of F. culmorum during 1989 to the warm dry summer. The incidence of F. nivale fluctuated during the 1988 and 1989 seasons, particularly during spring. The ability of F. nivale to produce ascospores and complete its infection cycle relatively rapidly in spring temperatures, accompanied by the natural death of late-formed tillers of winter wheat between March and the end of May is discussed as possible contributory factors to this.

A382

DEVELOPMENT OF *COLLETOTRICHUM GRAMINICOLA* ON WOUNDED MAIZE STEM TISSUES AS AFFECTED BY "WOUND HEALING" AND MAIZE GENOTYPE. A. Muimba-Kankolongo and G. C. Bergstrom, Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

This study was conducted to determine the effects of two types of resistance, maize genotype and wound healing, which each reduce anthracnose stalk rot, on early stages of fungal development at wound sites. Pith tissue cylinders (2 mm diam.) from the first internode above the brace roots of Cornell 281 (susceptible) and B 37 x LB 31 (resistant) plants at anthesis were sliced into 30 μm-thick discs and placed on glass slides in moist chambers. Then the wounded surfaces were inoculated with 10^6 conidia/cm^2 either immediately or 2 hr after the slices were made. Conidial germination at 6 hr after inoculation was 98% on freshly wounded Cornell 281 vs. 84% on aged wounds, and 63% on freshly wounded B 37 x LB 31 vs. 19% on aged wounds. Germ tube and appressorium development as well as cell penetration were delayed on aged wounds and in the resistant hybrid. "Wound healing" and genotypic resistances are expressed at early stages of the host/pathogen interaction and may involve a diffusable, antifungal substance.

A383

RICE DEAD TILLER SYNDROME IN ARKANSAS. Fleet N. Lee. University of Arkansas, P.O. Box 351, Stuttgart, AR 72160.

"Dead Tiller Syndrome" (DTS) was observed in rice cultivars in Northeast Arkansas. Symptoms first appear 6 to 10 days after the permanent flood and progress over 8 to 14 days until the flood water equilibrates to ambient soil and air temperatures. The initial symptom is slight discoloration of plants with some wilting. Advanced symptoms include severe wilting. Occasional plants have yellow to orange chlorosis along older leaf tips or leaf margins. Plants have a rotting culm which frequently produces a distinct odor when crushed. Decay begins internally at a node and can be well advanced before detection. The plant dies in 24 to 48 hours. The main culm in a hill is usually diseased. Tillers or nearby plants are not affected. DTS continues to develop throughout the growing season in areas where cold flood water is added to the field. A fungus from diseased tissue inoculated into 3 to 4 leaf stage rice seedlings caused culm rotting and death.

A385

STUDIES ON RHYNCHOSPORIUM SECALIS AS A SEEDBORNE PATHOGEN. A. D. Martinez-Espinoza, M. E. Bjarko and J. H. Riesselman. Dept. of Plant Pathology. Montana State University, Bozeman 59717.

Seed to seedling transmission was examined for Rhynchosporium secalis, the cause of scald on barley. Infected seed was obtained using a new artificial inoculation method. One ml of a suspension containing 150,000 spores was injected into the boot. Infected seed had the characteristic double-eyed shape of naturally infected seed. This method was highly effective in greenhouse and field tests. Transmission of the pathogen from seed to seedling was demonstrated in greenhouse tests. Between 33% and 41% of infected seed gave rise to infected seedlings. Symptoms were detected mainly in the first leaf (60%) or second leaf (30%). Conspicuous symptoms were observed sixteen days after emergence. These data conclusively demonstrate the importance of seedborne inoculum in the initiation of barley scald.

A387

EFFECT OF MELOIDOGYNE CHITWOODI ON THE GROWTH OF WHEAT. G. D. Griffin. USDA-ARS, Forage and Range Research, Utah State Univ., Logan, UT 84322-6300.

Populations of the Columbia root-knot nematode, Meloidogyne chitwoodi, from Utah (MC1) and Idaho (MC2) reduced (P<0.05) the growth of spring and winter wheat. There were less (P<0.05) tillers per plant on winter wheat inoculated with MC1 than MC2, although no differences were observed in the shoot dry weights. There were differences (P<0.05) in shoot dry shoot weight of spring cultivars; 'Fremont' was less (P<0.05) than 'Borah', 'Fielder' and 'Twin' at 24 ± 2 C. Differences in the nematode reproductive rate 'R' (Pf/Pi) were observed among nematode populations on winter wheat, but not among spring wheat. The greatest 'R' was observed with MC1 on 'Wanser' and 'Nugaines', whereas the smallest 'R' occurred with MC1 and MC2 on Daws wheat.

A388

PHENOLOGY OF ASCOSPORE RELEASE BY MYCOSPHAERELLA GRAMINICOLA FROM WHEAT STUBBLE IN RELATION TO CROP CYCLE, AND OTHER ASSOCIATED FUNGI. R. B. Madariaga and D. G. Gilchrist. Dept. of Plant Pathology, University of California, Davis, CA 95616

The objective of this study was to characterize the ascospore discharge of Mycosphaerella graminicola in relation to the crop cycle and to determine the survival ability of the spores and significance as primary inoculum. Wheat stubble, heavily infected with the Septoria Leaf Blotch pathogen, was sampled weekly in 1987-89. Sampled tissue was induced to discharge ascospores in humid water agar chambers. M. graminicola was detected first on December 14, 1987; November 28, 1988 and October 3, 1989. M. tassiana, Pleospora spp., Lepthosphaeria spp. and several species of yeast-like organisms expressed a characteristic release curve. In each season the peak discharge, February, was coincident with growth stage 25 to 39 (Zadoks) in early planted wheat. Severe infection followed 15 to 21 days later on lower leaves. In the absence of functional pycnidiospores the teleomorphic propagules appear to operate as the primary inoculum of the disease in California.

A389

SEVERITY OF WHEAT STREAK MOSAIC (WSM) ON WHEAT INOCULATED AT DIFFERENT PHYSIOLOGICAL MATURITIES. R. M. Hunger, J. L. Sherwood, and C. K. Evans, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947.

Hard red winter wheat cultivars (cvs) 'Chisholm' and 'Rall' are considered susceptible and resistant, respectively, to WSM. These cvs were planted on 12 Sep 88, 12 Oct 88, and 09 Nov 88, and were inoculated with wheat streak mosaic virus on 24 Mar 89 as described previously (Phytopath. 1988. 78:1503). At inoculation, plants of both cvs at each planting date were at Zadoks growth stage 31, 30-31, and 30 (Weed Res. 1974. 14:415-421). Development of WSM was monitored by symptomatology and enzyme-linked immunosorbent assay, and yield and thousand kernel weight were determined. Wheat which was physiologically young at inoculation showed severe WSM and yield reductions. Wheat inoculated at a more mature growth stage (Sep and Oct planting dates) exhibited mild WSM and inconsistent yield reductions. This suggests that planting date affects WSM severity on wheat infected in the spring.

A390

GENETIC RELATIONSHIPS AMONG *PHYTOPHTHORA INFESTANS* POPULATIONS FROM EUROPE, NORTH AMERICA, AND JAPAN. L. J. Spielman, W.-K. Gu, and W. E. Fry. Cornell University, Ithaca, NY 14853

We determined genotypes at the isozyme loci *Gpi1* and *Pep1* in collections of *Phytophthora infestans* from central and northern Mexico, the United States, Japan, The Netherlands, and Poland. The central Mexican sample had the highest diversity, as measured by the number of dilocus genotypes and the average number of alleles per locus. All collections had the same most common allele for both *Gpi1* and *Pep1*, but there were several alleles unique to particular regions. Non-random associations, consistent with a lack of sexual recombination, were found in the United States, where only the A1 mating type occurs, and also in northwestern Mexico and Japan, where both mating types occur. A comparison of samples from the early and late 1980's suggests that the genetic makeup of *P. infestans* in Europe has changed significantly during the last decade.

A391

DNA FINGERPRINTING IN *PHYTOPHTHORA INFESTANS*. S. B. Goodwin and W. E. Fry. Department of Plant Pathology, 334 Plant Science, Cornell University, Ithaca, NY 14853.

Two dispersed, moderately repetitive clones have been isolated from *Phytophthora infestans*. Each of these clones reveals 15 or more bands when hybridized to Southern blots of genomic DNA cut with the restriction enzyme Eco RI. Most of the bands are highly polymorphic, segregate as normal Mendelian markers, and do not appear to be tightly linked. Furthermore, individual banding patterns are stable through single-zoospore isolations. These clones may therefore provide true genetic fingerprints that are specific to particular individuals. Over 200 isolates collected from several *P. infestans* populations worldwide have been probed with these two clones, and differences were found among isolates that were identical for other markers. In some populations, where both mating types occur, reproduction is exclusively asexual, while in central Mexico almost every isolate is unique.

A392

VARIATION OF PLOIDY OF *PHYTOPHTHORA INFESTANS* IN CENTRAL AND NORTHERN MEXICO. Weikuan Gu, L. J. Spielman, J. M. Matuszak and W. E. Fry. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Ploidy of isolates of *Phytophthora infestans* with a variety of isozyme genotypes and A1, A2, and self-fertile mating types from central and

northern Mexico was tested with a simplified DAPI (4',6- diamidino-2-phenylindole) staining method. The isolates are mainly diploid, but great variation in levels of the ploidy, including apparent diploid, triploid and tetraploid, were found in both central and northern Mexico populations. In northern Mexico the genotype *Gpi* 100/100, *Pep* 92/100 was mainly diploid, while the genotype *Gpi* 100/111/122, *Pep* 100/100 included different ploidy levels. In central Mexico, the isolates primarily are diploid, but some are of higher ploidy.

A394

CHARACTERIZATION OF A CLASS III GENOMIC CLONE FROM *PSEUDOMONAS SYRINGAE* PV. *PHASEOLICOLA* WHICH SUPPRESSES PHASEOLOTOXIN$^-$ MUTANTS WHEN PRESENT IN MULTIPLE COPIES. K. Rowley, D. Clements, and S. S. Patil. Biotechnology Program, University of Hawaii, 3050 Maile Way, Honolulu, HI 96822.

Pseudomonas syringae pv. *phaseolicola*, the causal agent of halo blight of beans produces an extracellular toxin, phaseolotoxin, which is a potent inhibitor of ornithine carbamyltransferase. Previously, it was shown that EMS, UV, & Tn5 toxin minus mutants (Tox$^-$) were suppressed by 13 clones which could be divided into 3 apparently heterologous classes. One clone from Class III was characterized by Tn3HoHo1 mutagenesis, subcloning and sequencing. Interposon mutagenesis of the active region of the insert and its marker exchange into the wild-type genome showed that when present in a single copy, it fails to suppress toxin production in the wild type.

A395

GENES FOR RESISTANCE TO *PUCCINIA STRIIFORMIS* IN THE WHEAT CULTIVAR TRES. X. M. Chen and R. F. Line. USDA/ARS, Dept. of Plant Pathology, Washington State Univ., Pullman, WA 99164.

Seedlings of parents and F1, F2, and BC1 progeny from crosses of Tres with 12 cultivars used to differentiate races of *Puccinia striiformis* in North America and nine additional cultivars with known stripe rust resistance genes were tested for resistance to selected North American races. The 21 cultivars crossed with Tres have genes *Yr1-Yr10* plus 13 unnamed genes for resistance. Genetic analyses show that Tres has two resistance genes, one dominant and the other either dominant or recessive depending upon the cultivar crossed with Tres. Tres has no genes in common with 20 of the cultivars. Tres has a gene that may be allelic or closely linked with a resistance gene in Yamhill. The gene for resistance to race CDL-29 is different from the gene for resistance to race CDL-20 or gene for resistance to race CDL-27. Resistance to races CDL-20 and CDL-27 may be controlled by the same gene. Therefore, at least one of the genes in Tres is different from previously identified genes.

A396

RADIATA PINE RESISTANT TO DOTHISTROMA NEEDLE BLIGHT - BREED DEVELOPMENT AND EXPECTED REDUCTION IN DISEASE LOSSES. S.D. Carson, Forest Research Institute, Private Bag 3020, Rotorua, New Zealand.

A population of radiata pine (*Pinus radiata* D. Don) resistant to Dothistroma needle blight, caused by *Dothistroma pini* Hulbary, has been developed using methods which assume quantitative inheritance of resistance. Resistance exhibits high additive variance, moderately high heritability, and stability over sites and years. The 12% reduction in percent of needles infected, which is predicted on the basis of genetic theory, may be conservative because of the epidemiological effect of planting only resistant trees in a stand. In the presence of high Dothistroma infection, wood volume production has been greater for the resistant breed than for improved populations which have not been selected for resistance.

A397

GENETIC FINE STRUCTURE OF A COMPLEX RUST RESISTANCE LOCUS IN MAIZE. S. H. Hulbert and J. L. Bennetzen. Kansas State University, Manhattan KS, 66506 and Purdue University, West Lafayette IN, 47907.

Chromosome 10 of maize carries a cluster of genes which control resistance to the rust species Puccinia sorghi and P. polysora. RFLP markers which closely flank this region were mapped and used to study the genetic arrangment of this region. Most of the resistance factors mapped within about 0.2 map units of each other. Others, such as $Rp1^G$ and Rp5, mapped up to three map units away from the cluster. Recombination frequencies between Rp genes depended heavily on the parents used in the cross. Changes in resistance of progeny from some Rp heterozygotes arose by mechanisms other than simple recombination. Possible mechanisms are being investigated.

A398

LENGTH HETEROGENEITY OF rDNA CODING REGIONS IN RHIZOCTONIA SOLANI. Dolores Gonzalez and Rytas Vilgalys. Department of Botany, Duke University, Durham, NC 27706.

Length variation within the non-transcribed regions of rDNA is frequently observed both at the species and population level in many fungi. We employed sequence data and restriction analyses using the polymerase chain reaction to examine length variation within genic regions coding for both 18S and 25S RNA. Length polymorphisms were detected among different anastomosis groups of the *R. solani* complex for almost every region of the 25S RNA examined. Additional survey of 12 isolates from AG 1 also detected 25S RNA length variation between individuals. Several AG 1 isolates were found to possess up to 3 rDNA length variants. Sequence data and restriction analysis showed that most of the major length variation is located about 600 bp from the 5' end of the 25S RNA coding region, and extends up to 500 bases inside the gene. In contrast to the 25S RNA cistron, sequence analysis of 1200 bp from the 5' end of the 18S RNA gene showed no length variation and only base substitutions. The presence of genic rDNA length polymorphisms among and within isolates adds a new level of subgenomic variation that needs to be considered in comparative analysis of this gene.

A399

IN VITRO INDUCTION OF PSEUDOTHECIA, ASCOSPORE RELEASE AND VARIATION IN FERTILITY AMONG GEOGRAPHIC ISOLATES OF *LEPTOSPHAERIA MACULANS*. A. Mengistu, S.R. Rimmer, and P.H. Williams. Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

The sexual compatability and reproductive capacity in *Leptosphaeria maculans* from Europe, Canada, and Australia was assessed. To induce sexual mating pairs of single ascospore isolates were placed 1 cm apart on V8 agar in plastic petri plates, sealed with parafilm and incubated at 24 C under continuous fluorescent light (60-75 $\mu E\ sec^{-1}\ m^{-2}$). After 7 days, the developing fungal colonies are intermingled, and a layer of 2% water agar cooled to 55 C is poured over the V8 agar layer. Plates are resealed and incubated for 3-4 weeks at 16 C under black lights (Sylvania 40 watt, BLB) with a 12 h photoperiod. Release of ascospores was facilitated by placing a drop of 5% β-glucuronidase and water over the asci. There was a significant variation among isolates in the number of perithecia. Isolates from the different geogrphic areas were able to intermate. Isolates PHW 1275, 1276, and 1306, all from Australia, had the highest number of perithecia and were the most fertile of the isolates tested.

A400

EXPRESSION OF THE TRANSPOSABLE ELEMENT Tc1 IN THE NEMATODE *CAENORHABDITIS ELEGANS*. Anthony Radice and Scott Emmons, Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, New York 10461.

In order to understand the mechanisms underlying Tc1 excision and transposition, we are studying transcription and translation of the Tc1 open reading frame. In Northern blot hybridization experiments, we have shown that a Tc1-open reading frame probe hybridizes to a large number of RNA species, including ribosomal RNA. To further characterize Tc1 transcripts,

we have carried out PCR amplification experiments after first strand cDNA synthesis from polyA+ RNA preparations. We determined that there was a region of Tc1 that could not be amplified in this way, suggesting the presence of a 5' end. So far, the largest region we have amplified spans from nucleotide 304 to 1548 (putative polyadenylation signal). To study expression of the Tc1 ORF protein, we have three antisera that recognize the putative transposase, TcA. The antisera react with the TcA portion of a TrpE-TcA fusion protein isolated from *E. coli*, but not to a protein of the expected molecular weight in preparations of nematode proteins. Therefore, the Tc1 ORF protein, if it is expressed, is a rare protein.

A402

RELATIONSHIP AMONG THE VASCULAR WILT FUSARIA OF THE CHENOPODIACEAE. R. D. Martyn, D. H. Kim, C. M. Rush, and E. A. Dillard, Department of Plant Pathology and Microbiology, Texas A&M University, College Station 77843.

Isolates of *Fusarium oxysporum* causing vascular wilt in the Chenopodiaceae plants sugar beet (*Beta vulgaris*), spinach (*Spinacia oleracea*), and redroot pigweed (*Amaranthus retroflexus*) were compared using host pathogenicity, isozyme relatedness, and mtDNA RFLP patterns. Pathogenicity tests indicated a range of host specificity among the isolates, i.e. some were specific to their original host, a few primarily were pathogenic to their original host but caused some wilt on other hosts, and two isolates were highly pathogenic to both sugar beet and spinach and moderately pathogenic to pigweed. Isozyme profiles and mtDNA hybridizations correlated with the pathogenicity results. Isolates specific to sugar beet had similar isozyme matching distances, but were distant from isolates specific to spinach, while a cross-over isolate had a matching distance in between. RFLP analysis revealed three main polymorphic groups and two subgroups: isolates specific to sugar beet and spinach separated into two distinct groups while two cross-over isolates were in a third group. These data suggest that while most isolates display a high degree of host specificity, there exists within the population isolates that cross-over to other species within the Chenopodiaceae.

A403

CHARACTERIZATION OF ANASTOMOSIS GROUPS OF BINUCLEATE RHIZOCTONIA FUNGI USING RESTRICTION ANALYSIS OF RIBOSOMAL RNA GENES. M.A. Cubeta, E. Echandi, R. Vilgalys*, and T. Abernethy, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695 and Dept. of Botany, Duke University*, Durham, NC 27706.

Seven US and 15 Japanese binucleate Rhizoctonia anastomosis tester isolates were characterized by restriction analysis of ribosomal RNA genes. Total genomic DNA was extracted and a 1.4 kb fragment coding for 25S rRNA was amplified using the polymerase chain reaction. Amplified DNA was digested with six different restriction enzymes to determine restriction fragment length polymorphisms. Restriction patterns of CAG1 and AGD were similar to each other with all enzymes tested, but distinct from all other groups. Isolates belonging to CAG2, CAG3, CAG6, AGA, AGE, AGF and AGG had very similar restriction patterns but could be differentiated with a specific restriction enzyme. These findings support previous separation of binucleate Rhizoctonia fungi based on hyphal anastomosis by Burpee and Ogoshi.

A404

FUNGISTATIC COMPOUNDS FROM SOIL INHIBIT GERMINATION OF COCHLIOBOLUS VICTORIAE CONIDIA. J. A. Liebman and L. Epstein, University of California, Berkeley, CA 94720.

Conidia of the fungus Cochliobolus victoriae do not germinate on soil but do germinate on sterile distilled water. We investigated the cause of this soil fungistasis. To quantify fungistasis, we counted percentage germination of conidia. Conidia were incubated on agarose blocks which were placed on soil. The blocks were kept sterile and separate from soil by polycarbonate membranes with 0.2-μm pores. Thinner agarose blocks became fungistatic more quickly than thicker blocks, and blocks became more fungistatic with increasing time on soil. When removed from soil, blocks remained fungistatic, but only for a few hours. Agarose separated from soil by a glass cover slip and placed in an air-tight chamber did not become fungistatic. Four soils gave similar results. The data suggest that many soils contain a fungistatic compound which diffuses through agarose and which is not highly volatile.

A406

A NOVEL DIFFERENTIAL MEDIUM FOR THE QUANTIFICATION OF PHOMA TERRESTRIS IN ORGANIC SOILS. N. E. Strobel and J. W. Lorbeer, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Phoma terrestris, causal agent of pink root of onion, colonizes and stains cotton fibers a distinctive pink to red color. Cheesecloth, therefore, was employed as the basis of a novel differential medium for P. terrestris. Twenty ml of medium containing 3 g $NaNO_3$, 1 g $MgSO_4.7H_2O$, 0.5 g chloramphenicol, and 20 g agar, per liter of H_2O was poured over a 9-cm diameter single layer of sterilized cheesecloth in a plastic petri dish. Pink to red areas appeared on the cheesecloth in dilution plates of organic soils naturally infested with P. terrestris. The presence of the pathogen in these areas was confirmed by reisolation and pathogenicity tests on onion seedlings. This medium has been found to be useful for monitoring the influence of soil fumigation on populations of P. terrestris in the organic soils of commercial onion fields in New York.

A407

EFFECT OF PHOSPHORUS ON ASPARAGUS GROWN IN A PEAT-BASED MYCORRHIZAL INOCULUM. C.T. Pedersen[1], G.R. Safir[1], S. Parent[2] and M. Caron[2]. [1]Michigan State University, East Lansing, MI 48824 and [2]Premier Research Center, Rivière-du-Loup, Quebec G5R 4C9, Canada.

Field grown asparagus preinoculated with a commercial peat containing vesicular-arbuscular mycorrhizal (VAM) fungi Glomus intraradices (GI) and G. versiforme (GVR) previously exhibited improved survival and growth compared to non-inoculated plants. To determine if the VAM effect was nutritionally mediated, we evaluated the response of asparagus to different levels of applied P when grown in the greenhouse in a peat-based mycorrhizal inoculum. Dry weight, tissue P concentration and root colonization were assessed at 10 and 14 weeks. Plant growth increases by GI and GVR were positively correlated with root colonization. There was no significant response of plant dry weight to 100 ml of P solution applied to 10 cm pots weekly at 0, 50, 100 and 150 ppm for either inoculated or un-inoculated plants.

A408

VIRULENCE OF RHIZOCTONIA SOLANI AND OTHER RHIZOCTONIA ON POTATO AT THREE TEMPERATURES. D. E. Carling, and R.H. Leiner. Univ. of Alaska, 533 E. Fireweed, Palmer, AK 99645.

Pathogenicity of 59 isolates of Rhizoctonia from various geographical and host sources was determined on potato at 10, 15.5, and 21.1 C. Isolates representing 11 anastomosis groups of R. solani and other multinucleate and binucleate of Rhizoctonia were included. Isolates of R. solani AG-3 killed significantly more sprouts per plant at all temperatures than isolates of any other group. Damage to sprouts attacked by isolates of AG-3 was greater than damage caused by any other groups at 10 C, but isolates of AG-8 and AG-3 caused similar damage to roots at 10 C. At higher temperatures isolates of many groups were more virulent, particularly AG-5 and AG-4, but virulence of isolates of AG-3 and

AG-8 did not increase with temperature. Although isolates of AG-8 were as damaging as isolates of AG-3 to roots at all temperatures, AG-8 caused only minor damage to shoots. No groups were more virulent to shoots or roots at any temperature than AG-3. However, isolates of AG-8 cause severe damage to roots and may be able to cause economically significant reductions in yield.

A409

USE OF A HIGHLY SUSCEPTIBLE WATERMELON CULTIVAR TO SELECTIVELY RECOVER *FUSARIUM OXYSPORUM* F. SP. *NIVEUM* FROM SOIL FOR RACE DETERMINATION. D. L. Hopkins and R. J. Lobinske, Central Florida Research and Education Center, University of Florida, Leesburg, FL 34748.

Florida Giant, highly susceptible to all races of *Fusarium oxysporum* f. sp. *niveum*, was used to selectively recover the pathogen from soil. The three vegetative compatibility groups (VCG) of this Fusarium wilt fungus, one corresponding with the highly aggressive race 2, were mixed in various proportions in steam-sterilized soil. The pathogen was then isolated from Florida Giant seedlings that wilted when planted in this infested soil. The proportions of the three VCG isolated from wilted seedlings were very similar to the proportions originally blended in the soil. For example, when the VCG were combined 1:1:1, the recovered proportions were 28:40:32. This method appears to be suitable for determining the proportion of *F. oxysporum* f. sp. *niveum* in the soil that is the highly aggressive race 2. Using this technique, the resistant cultivar Calhoun Gray was shown to select for race 2 of the pathogen.

A410

SUPPRESSION OF APHANOMYCES DAMPING-OFF OF SUGAR BEET BY INCORPORATION OF GREEN PLANT RESIDUE INTO SOIL. Carol E. Windels and Donna J. Nabben-Schindler, Northwest Experiment Station, University of Minnesota, Crookston 56716.

Soils naturally infested with Aphanomyces cochlioides were collected from two fields and planted in the greenhouse (18 C) to 18 crops representing the Chenopodiaceae, Cruciferae, Gramineae and Leguminosae families. After 4 wk, plants were cut at soil level, dried at 38 C, cut into pieces, incorporated into the same soil from which the crop had been removed, and incubated for 3 wk. Soils then were planted with sugar beet (24-27 C). Incorporation of green oat residue resulted in an increase in sugar beet stand in both soils (86 and 83%) compared to sugar beet after sugar beet (4 and 55%) or sugar beet after fallow (2 and 23%). Root rot indices (0-100 scale) were decreased in both soils treated with green oat residue (17 and 16) compared to sugar beet after sugar beet (97 and 51) and sugar beet after fallow (98 and 82). Incorporation of green oat residue into soil may be useful in managing Aphanomyces damping-off of sugar beet.

A411

THE DISTRIBUTION OF ^{14}C IN PLANT TISSUE AND ROOT EXUDATE OF CITRUS INOCULATED WITH *FUSARIUM SOLANI*, *PHYTOPHTHORA CITROPHTHORA*, AND BOTH *F. SOLANI* AND *P. CITROPHTHORA*. L.-M. Dandurand and J. A. Menge. Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521

Heat-stressed and non-stressed citrus seedlings were labelled with ^{14}C for 48 hr, 6 wk after inoculating with *Fusarium solani* (Fs), *Phytophthora citrophthora* (Pc), or both Fs and Pc. At harvest, the distribution of ^{14}C among leaf, stem, root, and root exudate was measured by liquid scintillation. The percentages of ^{14}C in the leaf and stem fractions of plants inoculated with Pc were significantly greater than for non-inoculated plants. The percentages of ^{14}C in roots and root exudates were significantly less for plants inoculated with Pc. Fs did not have a significant influence on partitioning of ^{14}C. The percentages of ^{14}C in the leaf and root tissues of heat-stressed plants were not significantly different than those of non-stressed plants. Heat-stressed plants co-inoculated with Fs and Pc had a significantly lower percentage of ^{14}C in the root exudates than non-stressed, co-inoculated plants.

A412

PSORALEN COMPOUNDS ACTIVATE DISEASE RESISTANCE RESPONSE GENES IN PEA. A. Parsons, D. Horovitz, and L. A. Hadwiger, Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

Natural plant psoralen compounds applied to pea pods and subsequently cross-linked to pea DNA with 366 nm U.V. light have been previously shown to be elicitors of pisatin in peas. They also enhance a pattern of major proteins very similar to the pattern of proteins synthesized when peas are induced to resist the pathogen *Fusarium solani* f. sp. *pisi*. We now report that a synthetic psoralen, trioxsalen, 4'-amino methyl HCl (AMT), induces two PR-protein-coding genes, DRRG49 and DRRG206. Pea pod tissue was treated with AMT (60 μg/ml) for 45 minutes and subsequently cross-linked by U.V. 366 light for 12 minutes to initiate the induction observed. The DNA specificity of the psoralen binding in relationship to the mode of action of AMT and a biotic DNA-specific elicitor, chitosan, will be discussed.

A413

MOLECULAR CLONING OF THE TOX LOCUS FROM *COCHLIOBOLUS CARBONUM*. J.D. Walton, J.S. Scott-Craig, and J.-A. Pocard, DOE-Plant Research Laboratory, Michigan State University, E. Lansing, MI 48824.

We previously described the purification of two enzymes involved in the biosynthesis of HC-toxin, the cyclic peptide host-selective toxin from *C. carbonum* race 1 [Walton and Holden (1988) MPMI 1:128]. Antibodies against HTS-1, the enzyme that activates L-Pro and epimerizes it to D-Pro, were used to screen a cDNA library constructed in lambda gt11. An immunopositive phage insert of 1.1 kb hybridized to a synthetic oligonucleotide corresponding to the amino acid sequence of a tryptic peptide from HTS-1. The DNA sequence of the clone encoded additional amino acid sequences present in the protein. This DNA was present in all tox^+ isolates and absent in all tox^- isolates of *C. carbonum* tested. In a genetic cross between tox^+ and tox^- isolates, presence of this DNA segregated with the tox^+ phenotype. We conclude that the TOX locus in *C. carbonum* encodes the enzymes that synthesize HC-toxin and that HC-toxin production is associated with a DNA insertion or deletion event.

A414

CHARACTERIZATION OF THE TOX LOCUS OF *COCHLIOBOLUS CARBONUM*. D.G. Panaccione, J.S. Scott-Craig, J.-A. Pocard, and J.D. Walton, DOE-Plant Research Laboratory, Michigan State University, E. Lansing, MI 48824.

HC-toxin synthetase I (HTS-1) is an enzyme involved in the biosynthesis of HC-toxin, the host-selective toxin produced by race 1 but not races 2 or 3 of *Cochliobolus carbonum*. Genomic DNA sequences homologous to a cDNA clone for HTS-1 are found only in race 1 isolates of the fungus. By chromosome walking in both directions from the HTS-1-encoding gene, we have identified and cloned a 22-kb region of DNA that is unique to race 1. Border sequences on either side of the race 1-unique region contain elements that are moderately repeated (ca. 20 to 100 copies/genome) in race 1 and race 2. Left and right border sequences have homology with one another. We anticipate that the 22-kb, race 1-unique region is a gene cluster encoding HTS-1 and additional enzymes involved in HC-toxin biosynthesis.

A415

Aspects of pathogenesis and host resistance in the interaction between *Venturia inaequalis* and apples. C. Valsangiacomo, M. Müller, B. Koller, and C. Gessler. Institute of Phytomedicine, 8092 ETH-Zürich, Switzerland.

V. inaequalis and apple establish a peculiar relationship where the fungus develops between the cuticular membrane and cells of the upper epidermis without penetrating any cell. TEM studies showed local degradation of host epidermal cell walls. Enzymes potentially involved in this phenomenon, such as cellulases and polygalacturonases, were found in fungal liquid cultures. Polygalacturonase was purified to homogenity and characterized both biochemically and immunologically. The activity of cellulolytic enzymes was also detected and further purification and characterization are in progress. A polygalacturonase inhibiting protein (PGIP) from uninfected apple leaves was purified and partially characterized. The possible role of cell wall degrading enzymes and of PGIP in the interaction between *V. inaequalis* and *Malus* will be discussed.

A416

ELICITATION OF SESQUITERPENOID CYCLASE AND SUPPRESSION OF SQUALENE SYNTHETASE ACTIVITY IN POTATO TUBER TISSUE. M. N. Zook and J. A. Kuc. Department of Plant Pathology, University of Kentucky, Lexington, Kentucky 40546

Arachidonic acid (AA), an elicitor of sesquiterpenoid phytoalexins from Phytophthora infestans, caused a 20-fold increase in sesquiterpenoid cyclase activity in potato tuber tissue 48 hr after application, as compared to untreated tissue. Squalene synthetase activity decreased by 90% in the same elicitor-treated tissue 12 hr after AA application. Elicitation of sesquiterpenoid cyclase activity and suppression of squalene synthetase activity were also observed after inoculation with Helminthosporium carbonum, a non-pathogen of potato, and compatible and incompatible races of P. infestans. These results indicate that elicitation of phytoalexin accumulation in potato involves coordinate regulation of two important enzymes in the acetate-mevalonte pathway.

A417

GLYCEOLLIN ELICITORS INDUCE MAJOR AND DISTINCTLY DIFFERENT RESPONSES IN LOCAL AND DISTAL SOYBEAN CELL POPULATIONS. T. L.

Graham and M. Y. Graham, Dept. of Plant Pathology, The Ohio State University, Columbus, OH 43210.

Infection of soybean tissues by Phytophthora megasperma f. sp. glycinea (PMG) releases free daidzein (DZ) and genistein (GT) from constitutive conjugates. DZ is a precursor of the phytoalexin glyceollin and GT is directly toxic to PMG. PMG wall glucan treatment alone increases DZ and GT conjugate pools; glyceollin accumulation is only 10% of this response. Analysis of discrete cell populations proximal and distal to the point of elicitor application has yielded additional information. All elicitors tested caused accumulation of glyceollin only in the uppermost cell layers of soybean cotyledons. However, biotic elicitors stimulated de novo accumulation of DZ and glyceollin, whereas abiotic elicitors released the precursor DZ from existing conjugates. In cell layers below the treated surface, glyceollin was not induced; instead, a six-fold accumulation of the DZ and GT conjugates occurred in response to either class of elicitor.

A418

THE EFFECT OF MYCOTOXIN FUMONISIN B, ON THE GROWTH AND DEVELOPMENT OF MAIZE CALLUS. M.A.J. van Asch, F.H.J. Rijkenberg, and T.A. Coutinho. Department of Microbiology and Plant Pathology, University of Natal, P.O. Box 375, Pietermaritzburg, 3200, South Africa

The phytotoxic effect of Fumonisin B, (FB1), a mycotoxin of *Fusarium moniliforme*, was tested using callus from the scutella of immature cobs of maize, *Zea mays*. The callus was grown on modified MS medium with the toxin added in different amounts (0, 0.1, 1.0, 10 and 100 mg FB1 per liter). Callus growth decreased as the concentration of toxin increased, resulting in a significant growth reduction at the highest toxin level (100 mg of FB1/l). Transmission electron microscopy studies showed an increased level of activity in the treated cells resulting in thicker cell walls and the occurrence of starch grains. It is postulated that this increased activity led to cell disorganisation and, finally, death of many cells. At the 100 mg FB1/l treatment, callus cells appeared to be dead, but regrowth studies revealed that most of the callus pieces had retained their viability, even though the growth rate of the callus was significantly slower than in all the other treatments. Callus, grown at all other concentrations, recovered fully, and at the end of the regrowth period, no difference could be demonstrated between the other treatments.

A419

HRP MUTANTS OF Pseudomonas syringae pv. tabaci ACTIVATE THE TRANSCRIPTION OF GENES ASSOCIATED WITH DISEASE RESISTANCE IN BEAN. **J. L. Jakobek, and P. B. Lindgren, Department of Plant Pathology, North Carolina State University, Raleigh, N.C., 27695.**

We have been studying the induction by bacteria of genes in bean (Phaseolus vulgaris L.) associated with disease resistance. Slot blot and Northern analyses were conducted using RNA isolated from inoculated bean leaves and cDNA probes for phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), chalcone isomerase (CHI), or chitinase. Transcripts corresponding to all four cDNAs are rapidly induced in the bean cultivar 'Red Kidney' after inoculation with Hrp mutants of Pseudomonas syringae pv. tabaci (Pst), even though visible hypersensitive responses are not seen after inoculation with these mutants. This induction is very similar to that which occurs after bean is inoculated with wild-type Pst with respect to the timing and level of gene transcription. The expression of PAL after inoculation by Hrp strains is not transient, as this transcript is still detected 120 hours post-inoculation. PAL and CHI are also induced in bean after inoculation with P. fluorescens and heat killed Pst cells, but not after inoculation with Escherichia coli.

A420

INOCULATION OF BEAN WITH HRP MUTANTS OF PSEUDOMONAS SYRINGAE PV. TABACI ALTERS SUSCEPTIBILITY TO P. S. PV. PHASEOLICOLA. **P. B. Lindgren and J. L. Jakobek, Department of Plant Pathology, North Carolina State University, Raleigh, N.C., 27695.**

Bean plants (Phaseolus vulgaris L.) that are inoculated with Pt11528X1, a Hrp mutant of Pseudomonas syringae pv. tabaci (Pst), may be more resistant to infection by P. s. pv. phaseolicola (Psp) than are plants which have not been inoculated with this mutant. Although Pt11528X1 does not elicit a visible hypersensitive response on bean, certain genes which are associated with plant disease resistance are induced after bean is inoculated with this strain. The bean cultivar 'Red Kidney' was simultaneously inoculated with Psp and Pt11528X1, or inoculated with Psp eight hours after being inoculated with Pt11528X1. In both situations the population of Psp increased by at least 3 log units, compared to an increase of 5 to 6 log units when plants were inoculated with Psp alone. Disease symptoms were also variable on plants inoculated with both bacteria, but were reduced when compared to symptoms seen when bean was inoculated with Psp alone.

A421

RECOGNITION MECHANISM IN MYCOPARASITIC SYSTEM. M. S. Manocha, Y. Chen, N. Rao, Department of Biological Sciences, Brock University, St. Catharines, Ontario, Canada, L2S 3A1.

Recognition by the mycoparasite, *Piptocephalis virginiana*, of its hosts (compatible and incompatible) and nonhosts occurs at least at two different levels, i.e., cell wall and protoplast surface. At the cell wall level, the mycoparasite recognizes the differences in sugar distribution pattern between the host and nonhost species and it attaches to the former and not to the latter. Attachment of mycoparasite to its host surface could be inhibited by N-acetylglucosamine, glucose and arabinose. These three sugars are the major components of an agglutinin present as two distinct bands of glycoproteins, observed in SDS-PAGE of the cell wall extract of host and not of nonhost. These sugars, however, do not effect the appressorium formation which probably is affected by the protein component of the agglutinin. At the protoplast level, the mycoparasite recognizes the differences between the compatible and the incompatible host species. The exact nature of recognition at the protoplast level is not clear, but it seems to involve the rejection of self in the incompatible interaction and the acceptance of non-self in the compatible interaction.

A422

ISOLATION OF cDNA SEQUENCES FOR 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE FROM POTATO TUBER. Choi, D. I., Ward, B. L., Bostock, R. M., Department of Plant Pathology, University of California, Davis 95616.

Induction of potato HMGR activity precedes the accumulation of the phytoalexins, lubimin and rishitin, which accumulate following treatment of tuber tissue with the fungal elicitor, arachidonic acid, and are associated with hypersensitive response of potato cultivars to incompatible races of Phytophthora infestans. Southern blot hybridization of potato genomic DNA with a highly conserved sequence from the 3' region of the HMGR gene from Arabidopsis thalliana detects four EcoRI restriction fragments each present in one to several copies per haploid genome. Forty-seven positive clones were isolated after screening 670,000 plaques of λgt11 potato tuber cDNA library by hybridization with the Arabidopsis probe. Eleven independent cDNAs were subcloned and mapped. Northern blots of total RNA from potato tuber tissue and young tomato fruit detected a similar size transcript in both plants after hybridization with the Arabidopsis probe.

A423

CROSS PROTECTION INDUCED BY *FUSARIUM OXYSPORUM* AGAINST *VERTICILLIUM* WILT IN FUSARIUM RESISTANT TOMATO. P. E. Jorge, W. R. Chaney and R. J. Green, Dept. of Forestry and Natural Resources, Purdue University, West Lafayette, IN.

The occurrence of cross protection (induced resistance) was tested with near isolines of Roma tomato cultivars that differed in absence or presence of a gene(s) for resistance to vascular wilt pathogens. *Fusarium oxysporum* f. sp. *lycopersici* (inducer) and *Verticillium dahliae* (challenger) interactions were studied. Tomato seedlings in their 3-4 true leaf stage were root dip inoculated in suspensions of 7×10^6 propagules/ml of each fungus. Plants were incubated for 21 d under controlled soil temperature of 22 C. *Fusarium oxysporum* induced resistance against *V. dahliae* in Fusarium resistant Roma seedlings when inoculated simultaneously with both fungi. Resistance was expressed as a statistically significant higher fresh weight, lower disease severity index and lower stem isolation rate of Verticillium for plants inoculated with both fungi (protected) compared to plants inoculated only with *V. dahliae* (unprotected).

A425

THE ROLE OF AGRICULTURAL LIGNICOLOUS BASIDIOMYCETES AS BIOCONTROL AGENTS OF SOILBORNE PATHOGENS. R. E. Baird[1], C. C. Dowler[3], A. W. Johnson[3], S. C. Phatak[2], and D. R. Sumner[1]; Departments of Plant Pathology[1], Horticulture[2], and USDA[3], Coastal Plain Expt. Stn., University of Georgia, Tifton, GA 31793.

Competition of lignicolous Basidiomycetes with Rhizoctonia solani AG-4 is not fully understood. Various conservation tillage cropping systems, near Tifton, GA, were studied to: 1) identify lignicolous Basidiomycetes present in the fields, 2) characterize population dynamics throughout the growing season, and 3) evaluate the individual species for their potential as biocontrol agents. During the 1989 growing season, ten major fungal species were identified in two fields (e.g. Coprinus plicatus, Marasmius siccus, and Sphaerobolus stellatus). The majority of fungi were observed in no-till treatments (30% or more debris on the soil surface). No lignicolous fungi were observed in treatments where residues were burned. One lignicolous species (Coprinus plicatus) protected snapbean plants from R. solani AG-4 in a preliminary greenhouse test.

A426

BIOCONTROL OF AMARANTHUS ALBUS WITH APOSPHAERIA AMARANTHI. A. S. Mintz, and G. J. Weidemann, Plant Pathology Dept., University of Arkansas, Fayetteville, 72701.

Growth chamber studies were conducted to determine the potential of Aposphaeria amaranthi as a biocontrol agent for tumble pigweed (Amaranthus albus L.). Seedlings at the 4-6 true leaf stage were killed within two days when inoculated with conidial suspensions of $1x10^6$ spores/ml when given a 12-hr dew period at 28 C. Conidial concentrations as low as $1x10^4$ spores/ml were sufficient for complete control when the dew period was increased to 24 hr. Dew temperatures ranging from 16 to 28 C were conducive for disease development. The onset of the dew period following inoculation could be delayed for 24 hr without an apparent decrease in disease severity. Field inoculations in 1989 resulted in 75% mortality of tumble pigweed seedlings. Results from these studies suggest that A. amaranthi may have potential as a bioherbicide for tumble pigweed.

A427

RATE OF SPORE MORTALITY FOR THE NEMATOPHAGOUS FUNGUS HIRSUTELLA RHOSSILIENSIS. B. A Jaffee, A. E. Muldoon, M. Mangel, and R. Phillips, University of California, Davis 95616.

Hirsutella rhossiliensis, an obligate parasite of nematodes, produces nonmotile spores which adhere to and infect passing hosts. Natural mortality (loss of adhesiveness) of spores was quantified in vials containing 17 cm^3 loamy sand (heated to 60 C for 2 hr, adjusted to -60 mbars matric potential) at 20 C. Vials contained no nematodes and were seeded with 1.1×10^5 spores at day 0. Vials were assayed periodically for spores by adding hosts (juveniles of Heterodera schachtii). Hosts were extracted after 3 days, and number of spores/host and infection of hosts were determined. Spore mortality was inferred from changes in spore detection. Each vial was assayed once (five vials/date). Some spores died within 21 days and others were viable for at least 200 days. Over 90% of the hosts with spores were infected regardless of date. The relative rate of spore mortality was 0.072 ± 0.003/wk.

A428

ENVIRONMENTAL FITNESS OF SELECTED ENDOPHYTIC BACTERIA: A POTENTIAL BIOCONTROL FOR OAK WILT. E. H. Gehring, D. N. Appel, C. F. Gonzalez, Department of Plant Pathology and Microbiology, Texas A&M University, College Station 77843; and T. H. Filer, USDA-Forest Service, Stoneville, MS 38776.

Three endophytic bacterial isolates with antibiotic resistance markers were introduced into mature live oaks (*Quercus fusiformis*) to evaluate their environmental fitness as potential biocontrol agents for oak wilt, caused by *Ceratocystis fagacearum*. In a previous test, one isolate (*Pseudomonas denitrificans*, strain 1-15) suppressed oak wilt development in containerized trees. Distribution of the introduced bacteria was determined by dissection of an injected tree at 1 wk. Strain 1-15 was recovered in 12% of the root, bole, and branch samples, while *Bacillus pumilus* (1-1) was found in only 3% of the samples. *P. putida* (Y-20) was not recovered from a dissected tree. Persistence of the bacteria in trees was determined by taking samples at 1 day, 1 wk, 1 mo, 3 mo, and 6 mo after introduction and plating tissue samples onto a medium amended with the appropriate antibiotic(s). Strains 1-15 (branch sample) and Y-20 (bole sample) were isolated at 1 and 3 mo, respectively, while all other samples taken did not yield any of the introduced isolates.

A429

DEVELOPMENT OF PUCCINIA CARDUORUM AND ITS IMPACT ON MUSK THISTLE IN VIRGINIA. A.B.A.M. Baudoin, Dept of PPWS, R.G. Abad, L.T. Kok, Dept of Entomology, VPI&SU, Blacksburg, VA 24061, and W.L. Bruckart, USDA-ARS, Frederick, MD 21701.

A Puccinia carduorum strain from Turkey was evaluated as a biological control agent for musk thistle (Carduus thoermeri) in a 2-yr field trial. Musk thistle plots were inoculated successfully with the rust fungus in the fall and spring. To assess the pathogen's effect on selected nontarget species (slightly susceptible in greenhouse trials), artichoke and selected Cirsium spp. were planted between the inoculated plots. One rust pustule was found on an artichoke plant in 1989; all other plants remained rust-free despite severe disease on surrounding musk thistles. On musk thistle, pathogen spread was limited during the rosette stage; disease became severe only when the plants bolted. The rust spread only a few hundred meters in 1988, but in 1989 traces of rust were detected at naturally occurring musk thistle stands up to 7 km away. Rust had little effect on plant size, but reduced seed production and accelerated senescence significantly in 1989.

A430

BIOLOGICAL CONTROL OF FUSARIUM MONILIFORME IN RICE BY ANTAGONISTIC BACTERIA. T.W. Mew and A.M. Rosales. The International Rice Research Institute, P.O. Box 933, Manila, Philippines

Bakanae disease of rice caused by Fusarium moniliforme is a seedborne disease. The potential of antagonistic bacteria from paddy water, rhizosphere soils and rice plants, to control this disease was assessed by dual-culture, blotter and seed germination techniques. Out of 441 isolates, 113 were inhibitory to mycelial growth of the pathogen. Bacterization of naturally-infected IR42 seeds reduced bakanae incidence from 5-48% and 67-96% in seedbox and seedbed tests, respectively. Bakanae incidence in IR58 was reduced by 33-84% in seedbox test. Using the blotter and seed germination tests, bacterial isolates were classified into three groups: 1) those that promoted germination and enhanced seedling vigor 2) no effect on germination and 3) those that are deleterious which inhibited germination.

A431

SUPPRESSION OF PHYTOPHTHORA ROOT ROT OF COWPEA BY BACTERIAL BIOCONTROL AGENTS. W.G.D. Fernando and R.G.Linderman, Oregon State University, and USDA-ARS Hort. Crops Research Lab., Corvallis, OR 97330

In greenhouse pot experiments, seeds of cowpea (Vigna unguiculata var. Blackeye) were planted in pasteurized sand-soil infested with Phytophthora vignae. Seeds were treated or not with suspensions of bacterial biocontrol agents (10^8 cfu/ml) known to inhibit the pathogen in vitro; some were isolated from cowpea field soils in Sri Lanka where the pathogen was present but the disease was absent. After 2 weeks, several bacteria had significantly increased plant survival and prevented disease symptoms. Though most bacteria increased plant dry weight compared to the control (pathogen only), the increases were only significant for two of the Sri Lankan isolates. These findings suggest that seed treatments with bacterial biocontrol agents could provide control of Phytophthora root rot of cowpea.

A432

VOLATILE INHIBITORS OF PHYTOPHTHORA SPECIES PRODUCED BY BACTERIAL BIOCONTROL AGENTS. R.G.Linderman, W.G. D.Fernando, and L.Pscheidt, USDA-ARS Horticultural Crops Research Laboratory, Corvallis, Oregon 97330.

Several root pathogen bacterial biocontrol agents inhibited several species of Phytophthora in vitro, including P. cinnamomi, P. cactorum, P. syringae, P. cambivora, and P. vignae, due to agar-diffusible substances, but some also inhibited all the P. spp. in divided dishes by means of volatiles. Isolates tested of Pseudomonas, Enterobacter, Serratia, and Bacillus produced volatile inhibitors, but Haffnia, Salmonella, Agrobacterium, Alcaligenes did not. Production of volatile inhibitors in vitro was substrate dependent, and no volatile inhibitors were produced by effective bacteria added to sterile soil unless some substrate was also added. These results demonstrate that production of volatile inhibitors by biocontrol agents should be considered as a potential mechanism of biocontrol of root pathogens.

A433

THE HYPERPARASITE AMPELOMYCES QUISQUALIS INCREASES YIELD AND PHOTOSYNTHESIS OF POWDERY MILDEW-INFECTED CUCUMBER AND ZUCCHINI. Abraham Sztejnberg and Shadad Abo-Foul. Dept. of Plant Pathology & Microbiology, Faculty of Agriculture, Rehovot 76100, Israel.

Effective biocontrol of cucumber (C) and zucchini (Z) powdery mildew (PM) was obtained by Ampelomyces quisqualis (AQ), applied as a suspension of 10^6 spores/ml, alone or with the fungicide pyrazophos. In one greenhouse trial, application of AQ alone to CPM significantly decreased disease severity and increased C yield by 50%. A similar increase was obtained by applying pyrazophos alone or with AQ. In a second trial, all chemical and AQ treatments increased yield over untreated, PM-infected controls, which failed to produce fruits. In a field trial, when treating ZPM with AQ or with any of 5 recommended fungicides, similar yields were obtained from the AQ treatment in all but one of the fungicides. In AQ-treated plants this yield was 39% higher than in the untreated control. The level of photosynthesis in AQ-treated CPM-infected, untreated CPM-infected, and healthy C plants was 10.2, 3.8, and 12.8 μmol CO_2 m^{-2} s^{-1}, respectively, i.e., 170% higher in the AQ-treated than in the CPM untreated plants.

A434

EFFECT OF *GLOMUS INTRARADICES* AND *MELOIDOGYNE ARENARIA* ON GROWTH OF PEANUT CULTIVAR FLORUNNER. R. K. Garber, J. L. Starr, and R. A. Taber. Department of Plant Pathology and Microbiology. Texas A&M University, College Station 77843.

Greenhouse and microplot studies were conducted to determine the interaction effect of *Glomus intraradices* (Gi) and *Meloidogyne arenaria* (Ma) on peanut growth. At 24 days after emergence, seedlings inoculated with Gi or Gi + Ma possessed greater shoot weights and leaf areas than seedlings inoculated with Ma or the controls. By 54 days, leaf areas and shoot weights were 3-fold greater in seedlings receiving either Gi or Gi + Ma. Mycorrhizal colonization did not inhibit nematode penetration or development during the first 24 days. However, when Gi colonization increased, during the period of 24-54 days after Ma inoculation, a reduction in the number of nematodes per root system was observed. Microplot studies revealed fewer Ma eggs/g root and fewer eggs/female in mycorrhizal root systems at 54 days. Yields of Ma infested plants were enhanced by inoculation with Gi.

A435

EFFECT OF SELECTED MARIGOLD CULTIVARS ON PENETRATION, DEVELOPMENT, AND REPRODUCTION OF *PRATYLENCHUS PENETRANS*. M. A. Arevalo-Guerra and M. P. Ko, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Studies of penetration, development, and reproduction of *Pratylenchus penetrans* (PP) were performed using marigold cultivars differing in suppressiveness to PP. Gold Galore (GG) and Pumpkin Crush (PC) were inoculated with 2000 PP (93% juveniles), and the total number and proportions of various developmental stages of PP in roots were monitored over a 7 week (WK) period. Wando pea, a good host for PP, was used as a control. PP penetrated 3-4 times as many pea roots as GG and PC roots after one wk. Proportions of juveniles in pea decreased from 90% at wk 1 to 29% at wk 4, concomitant with a 9% increase in adults. Proportions of juveniles or adults in PC followed a similar pattern but delayed in time compared to pea. In contrast, juveniles and adult proportions remained constant in GG at 90% and 8%, respectively. Proportions of eggs in pea and PC had similar patterns following the appearance of adults, reaching peaks at wk 5 and wk 7, respectively. Egg proportions in GG were nearly 0% at all times. The final Pf/Pi ratio was 9.5 in pea, 0.57 in PC and 0.04 in GG. It was concluded that marigold cultivars suppressed PP by decreasing root penetration, arresting or delaying normal development, and/or lowering egg production.

A436

A METHOD TO OBTAIN LARGE INDIVIDUAL NUMBERS OF EGGS, SECOND STAGE JUVENILES, OR ADULTS OF *PRATYLENCHUS PENETRANS*. M. P. Ko and M. Arevalo, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Separation of *Pratylenchus penetrans* (PP) into distinct developmental stages has been difficult. A method was here developed to obtain large individual numbers of eggs, second stage juveniles or adults. Roots of PP infected Wando pea seedlings were mechanically macerated in water and the resultant slurry was separated into residue (R_1) and filtrate (F_1) fractions with a 38 μ pore-size screen. Vermiform nematodes (VN) were recovered from the R_1 fraction by the pie-pan method followed by repeated passages (5 or more times) through the 95 μ pore size sieve to yield large number of adults (males and females) uncontaminated by juveniles. The F_1 fraction was concentrated by sedimentation to a minimal volume of water. Eggs along with smaller VN stages were recovered from the sediment at the water/sucrose interphase (I) by centrifuging the suspension onto a cushion of 20% w/v sucrose. Contaminating VN stages were removed from the eggs by repeatedly sieving through the 38 μ pore-size screen. Finally, eggs were collected on a 10 μ pore-size screen, rinsed with water, and used either immediately for experiments or set aside in a shallow dish of water to allow second stage juveniles to hatch. 30-50% of the latter hatched from the eggs during a one-week incubation period at 25°C. The final yields of the eggs and adults were both 1-5% of the sum of nematodes recovered from the slurry.

A437

GUANIDINE HYDROCHLORIDE AS SOIL AMENDMENT FOR NEMATODE CONTROL. Ru-Ju Chian and R. Rodriguez-Kabana. Department of Plant Pathology, Alabama Agricultural Experiment Station, Auburn University, Alabama 36849-5409.

In greenhouse experiments Guanidine hydrochloride (GuHCl) reduced larval populations of root-knot nematode (Meloidogyne arenaria) and cyst nematodes (Heterodera glycines) in soil and in root tissue of soybean. Larvae of cyst nematode were less sensitive to GuHCl than those of root-knot nematode. At concentrations between 0.1-0.15 gm/kg soil GuHCl was not phytotoxic and was highly effective in nematode suppression. GuHCl also stimulated plant growth at concentrations below 0.5 gm/kg soil in the presence of nematodes.

A438

SURVEY OF PLANT PARASITIC NEMATODES ASSOCIATED WITH TURFGRASS DAMAGE IN WEST VIRGINIA. J. B. Kotcon. Div. of Plant and Soil Sci., West Virginia Univ., Morgantown, WV 26506-6057

One hundred and twenty soil samples from fairways and greens of 8 WV golf courses were assayed for nematodes using centrifugal flotation extraction. Twelve species of plant parasitic nematodes and 5 predaceous nematode species were identified. Tylenchorhynchus agri and Hoplolaimus galeatus were most frequently associated with unthrifty growth and decline of turfgrass. In a few sites, Tylenchorhynchus dubious, Trichodorous proximus, and Longidorus breviannulatus were associated with damage, especially on bentgrass greens. Symptoms were most severe during July and August when high temperatures combined with stunted root systems to induce drought stress. Population densities of T. agri and H. galeatus were not reduced significantly by ethoprop at 27 kg. ai./ha 7 weeks after treatment and Criconemella curvata populations were greater than in untreated plots.

A439

SOYBEAN CYST NEMATODE RACES IN TENNESSEE. Lawrence D. Young, USDA-ARS, 605 Airways Blvd., Jackson, TN 38301.

A survey of soybean cyst nematode (SCN), Heterodera glycines, races present in Tennessee soybean fields was conducted in 1988. One sample was taken for each 20,000 hectares of soybeans grown in each county. SCN race determination based on reproduction on standard differentials plus reproduction on Bedford (race 14 resistance from PI 88788) and Cordell (race 5 resistance from PI 90763) soybean cultivars were measured in a greenhouse for populations from 21 fields. Races 2, 3, 4, 5, 6, and 9 represented 14%, 5%, 10%, 38%, 19%, and 14%, respectively, of the populations. The amount of reproduction (female index) on race differentials PI 88788 and PI 90763 was not a good indicator of the reproduction occurring on cultivars Bedford and Cordell. The female index for eight populations was < 10 on the PI's but was > 30 on Bedford or Cordell. Recommendations of cultivars to be planted should be based on SCN reproduction on the cultivars instead of reproduction on the race differentials.

A440

POPULATION DYNAMICS OF THE POTATO ROT NEMATODE ON SNAPBEAN. A. E. MacGuidwin and D. J. Wixted, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Ditylenchus destructor, the potato rot nematode (PRN), is usually detected only in potato tubers. To improve detection before planting potato, we studied the population dynamics of PRN on snapbean, a crop rotated with potato. PRN reproduced on intact plants grown in heat-treated and untreated field soil and in monoxenic root explant cultures. Nematodes functioned as both ecto- and endoparasites of roots and infected stems of intact plants, producing elongate lesions as high as 10 cm above the soil line. No organisms besides PRN were recovered from lesions on stems. Six wks after planting, the proportion of the population infecting stems varied from 1 to 34% in a series of experiments. Tubers showing dry rot symptoms typical of PRN were recovered from potato grown in soil infested with infected bean stems. In general, equal proportions of PRN populations were recovered from soil and roots.

A441

LOCATION AND SIZE OF SYNCYTIA INDUCED BY *Heterodera glycines* IN THE ROOT TISSUES OF TWO SOYBEAN CULTIVARS. Yum, K. J., Park, E. W., and Kim, Y. H. Department of Agricultural Biology, Seoul National University, Suwon, 440-744. Korea.

The soybean cultivars Bangsa and SNUA were compared in terms of location and size of syncytia within the root tissue after penetration of the soybean cyst nematode. The number of cysts reproduced on Bangsa and SNUA after inoculation in the greenhouse were 336 cysts (range: 177-513 cysts) and 253 cysts (range: 197-360 cysts), respectively, suggesting that these cultivars were equally susceptible. However, syncytium development in the root tissues incited by the soybean cyst nematode differed in the two cultivars. Transverse sections of roots at the sites of nematode

penetration showed that considerably larger area of the stelar region of root tissues of Bangsa (33.2%) was displaced by syncytia than that of SNUA (6.3%). Thus, logitudinal transport of water and nutrients would likely be more severely inhibited by syncytia in Bangsa than in SNUA. Although both Bangsa and SNUA were susceptible to *H. glycines*, SNUA may be more tolerant to damage caused by the soybean cyst nematode than Bangsa.

A442

HOST RESISTANCE MANIFESTED IN EGRESSED *GLOBODERA ROSTOCHIENSIS* JUVENILE INFECTIVITY. L. L. Porter and R. K. Horst, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Potato cultivars with the H_1 resistance gene to *Globodera rostochiensis* produce a greater emergence of second-stage juveniles (J2s) than their susceptible counterparts. An *in vitro* root assay was devised to determine the ability of H_1-egressed J2s to invade subsequent host roots. Roots of susceptible and resistant cultivars on Hussey and Stacey medium were inoculated with J2s collected from resistant (R) and susceptible (S) plants. Freshly hatched J2s from cysts maintained in an incubator (I) served as a control. Penetrations of roots by J2s were evaluated 48 h after inoculation. Treatment I showed the highest occurrence of penetration in susceptible roots. J2s from the S treatment retained some ability to penetrate susceptible roots. Egressed J2s from the R treatment did not penetrate either susceptible or resistant roots. These results suggest that H_1 resistance produces a population of egressed J2s with reduced ability to invade potato roots.

A443

HOST RANGE STUDIES OF PERONOSCLEROSPORA SORGHI (DOWNY MILDEW) IN NIGERIA. O.M. Olanya, J. M. Fajemisin, G.K. Weber, and S.K. Kim,, 1990. International Institute of Tropical Agriculture, PMB 5320, Ibadan, Nigeria

Several native grasses, sorghum varieties and maize genotypes were tested for susceptibility to Peronosclerospora sorghi using artificial inoculations at two locations in the South-West and North-Central parts of Nigeria. In the South-Western location, none of the native grasses or the sorghum varieties were infected, but most maize varieties were susceptible. In the North Central zone, sorghum genotypes Tx412, Tx430, ICMPES-28, SDS2298, FEBL, and most maize varieties were systemically infected. Among the native grasses, only Roettboelia cochinchinensis and Dactyloctenium aegyptium were partially susceptible. Oospores and conidiospores were observed in the North-Central savanna ecology while only conidial sporulation of downy mildew was detected in the South-Western forest ecology. The susceptibility of differential sorghum hosts to the North-Central but not to the South West Downy mildews may suggest the presence of two pathotypes in Nigeria. New maize varieties developed at IITA are highly resistant against downy mildews in both areas.

A444

Maixner, M., Pearson, R.C.: *Scaphoideus titanus* Ball, a possible vector of Grapevine Yellows Disease in New York. Dept. of Plant Pathology, Cornell University, New York State Agric. Exp.Stn., Geneva 14456

Scaphoideus titanus is the vector of Flavescence doreé (FD) of grape in Southern Europe. It was collected in New York vineyards of *Vitis vinifera* affected by Grapevine Yellows Disease (GYD), which has identical symptoms to FD, and in adjacent wild *Vitis riparia*. None of the wild grapes exhibited symptoms of GYD. Insect collections in spring and summer indicate a migration of adult leafhoppers from wild grapes into commercial vineyards. Feeding of collected leafhoppers on *Vicia faba* in a greenhouse resulted in yellows symptoms typical of infection with mycoplasma-like-organisms (MLO) in 29 % of the host plants within three to four weeks. Symptoms of GYD have not yet been detected in potted grapes which were fed upon by the same collection of leafhoppers. Individual leafhoppers were tested by ELISA using polyclonal antibodies to FD (supplied by Boudon-Padieu and Caudwell, Dijon, France). A positive reaction of 13.3 % of the tested insects from commercial vineyards and wild grapes indicates a serological relationship between the pathogen of FD and the MLOs in New York leafhoppers. MLOs were observed by ISEM in individual preparations of ELISA-positive leafhoppers.

A445

PURIFICATION AND CHARACTERIZATION OF ANTIGENIC FUNGAL GLYCOLIPIDS FOR SPECIFICALLY DETECTING *BOTRYTIS CINEREA* IN JUICE FROM INFECTED GRAPES. R.W. Ricker, R.M. Bostock, & J.J. Marois. Department of Plant Pathology, University of California, Davis, CA 95616.

Immunization of rabbits with crude extracts from *Botrytis cinerea* stimulated production of polyclonal antibodies that bind proteins and various glycoconjugates, with early immune response directed primarily against the carbohydrate portion of an extremely antigenic glycolipid (ABGL). ABGL is unique to *B. cinerea* and apparently integrated into membranes but readily solubilized with surfactants. Its presence in juice can be measured quantitatively by immunoassays (ELISA) and correlated to the degree of infection by *B. cinerea* in individual berries and grape clusters. ABGL is small (M_r < 700 daltons) and appears to have only one epitope available for binding with antibodies. The small size, univalent nature, and uniqueness make ABGL an ideal candidate for direct competitive immunoassays to specifically detect *B. cinerea*. In a highly purified state, however, ABGL cannot be adsorbed onto plastic and measured directly on the surfaces of microtiter wells using heterogeneous assays. Details concerning the structural modification of this molecule for use in immunoassays will be presented.

A446

EFFECT OF EARLY-SEASON POWDERY MILDEW ON FORMATION AND SURVIVAL OF TILLERS OF WINTER WHEAT. K. L. Everts, and S. Leath, USDA-ARS, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616.

Early-season powdery mildew severity and tiller formation was studied on three winter wheat cultivars differing in susceptibility and with or without triadimenol seed treatment. Plants in one-half meter of row/plot were destructively sampled four times during tiller formation to determine the initiation and survival of tillers at different nodes. Disease severities ranged from 0 to 11% on the last fully expanded leaf during tillering. Analyses of variance indicated that cultivar and seed treatment and the interaction of cultivar x seed treatment influenced disease severity and tiller formation. Previously reported yield increases associated with triadimenol seed treatment may be in part due to increased tillering or tiller survival. The correlations between mildew severity, tiller initiation and survival are presently being examined.

A447

PLANT GROWTH AND YIELD DEVELOPMENT OF SOYBEAN UNDER THE INFLUENCE OF PHAKOPSORA PACHYRHIZI. X. B. Yang, T. A. Tschanz, and W. M. Dowler. USDA-ARS, Frederick, MD 21701 and USDA-APHIS, Hyattsville, MD 20782.

Effects of Phakopsora pachyrhizi (causal agent of soybean rust) on growth and development of soybean were evaluated to integrate a disease model with a model simulating soybean yield. Disease reduced potential yield by decreasing the maximum pods/plant up to 40% at plant growth stage R6. From R6 to R7, abortion of pods was significantly higher for severely diseased plants than protected plants. Potential number of seeds/pod at R6 was not affected. Rates of seed growth (g/day) from R6 to R7 were reduced as much as 60% in diseased plants compared with protected plants. Dry matter was partitioned more to seeds in diseased plants than protected plants during the period from R6 to R7. The periods required for plant growth from R1 to R6 and R6 to R7 were reduced up to 9 and 5 days respectively in diseased plants.

A448

COMPARING EFFECTS OF PHAKOPSORA PACHYRHIZI ON INDIRECT AND DIRECT YIELD COMPONENTS, PLANT YIELD, AND PLOT YIELD OF SOYBEAN. X. B. Yang, W. M. Dowler, and T. A. Tschanz. USDA-ARS, Frederick, MD 21701 and USDA-APHIS, Hyattsville, MD 20782.

To determine the optimum coupling point to integrate a soybean rust model with a soybean simulation model, relationships of disease progression to yields of 6 soybean cultivars in two years were quantified at four levels: indirect yield components, direct yield components, plant yield, and plot yield. Indirect yield components were poorly correlated with disease progression. However, direct yield components were consistently correlated with disease progression for all the cultivars. Disease did not affect number of plants per plot. Plant yield and plot yield were significantly correlated with disease progression for all cultivars. Plot yields were accurately predicted using green leaf area duration from plant growth stages R6 to R7 and area under disease progress curve as a predictor. Results indicate that the disease effects were best determined at the plot yield level.

A449

EFFECT OF FROGEYE LEAF SPOT ON SOYBEAN YIELDS. K.E. Dashiell and C.N. Akem. Grain Legume Improvement Program, International Institute of Tropical Agriculture, Ibadan, Nigeria.

Three soybean (Glycine max) cvs., Samsoy 1, (susceptible), TGx 1025-12E (moderately resistant) and TGx 996-26E (resistant), were evaluated for yield losses resulting from frogeye leaf spot caused by Cercospora sojina. Replicated field plots were established at 2 locations in Nigeria that are naturally infested with C. sojina. The cvs were either not sprayed, sprayed once or twice during the growing season with the fungicide benomyl. Disease spread was early in the season from border rows of a susceptible soybean cv to the plots. Mean disease severity (DS) and disease incidence (DI) for unsprayed

cultivars ranged from 0.6 to 4.5 and 6.3 to 95%, respectively. Plots receiving 2 sprays had lower DS and DI values, ranging from 0.5 to 2.4 and 5.0 to 42.0%, respectively. Differences between unsprayed and double sprayed plots for yield and 300-seed weight, ranged from 2.5 to 58.8, and 0.6 to 28.6%, respectively. Seed weight was negatively correlated with DS and DI.

A452

INOCULATION AND EARLY DETECTION OF ANISOGRAMMA ANOMALA ON SEEDLINGS OF CORYLUS AVELLANA. J.K. Stone, K.B. Johnson, and *J.A. Pinkerton. Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902, and *USDA/ARS Hort. Crops Research Lab., Corvallis, OR 97331.

Hazelnut seedlings were inoculated with ascospores of A. anomala, the causal agent of eastern filbert blight. Sets of 20 inoculated trees were either placed immediately on an open bench or intermittently misted at 10 min. intervals for 3, 7, or 14 days. Infection was detected after 1-3 mo by examination of subperidermal sections for distinctive intracellular hyphae. In one experiment, all seedlings misted for 3, 7, or 14 days, and 38% of the nonmisted seedlings contained hyphae of A. anomala. In a second experiment, hyphae were detected in 4% of nonmisted seedlings, 34, 20, and 71% of those misted for 3, 7, or 14 days respectively. Initial germination was observed on cleared tissue 4-5 days following inoculation. Apparently, long periods of free moisture aid the infection process, but are not required for successful infection.

A453

INFLUENCE OF TEMPERATURE ON DEVELOPMENT OF PHYTOPHTHORA GUMMOSIS AND ROOT ROT ON CITRUS. M. E. Matheron and J. C. Matejka, Yuma Agricultural Center, University of Arizona, Yuma, AZ 85364

Studies were initiated to examine the effect of temperature on development of Phytophthora gummosis and root rot of citrus as well as the influence of temperature on sporulation of Phytophthora citrophthora and P. parasitica. Maximum production of sporangia by each fungus occurred at 25 C, while minimal sporulation occurred at 10, 15, and 35 C. Slight or no growth of lesions was observed when either intact stems or excised stem pieces of rough lemon were inoculated with P. citrophthora or P. parasitica and incubated at 5 and 30 C or 10 and 30 C, respectively. The suppressive effect of certain temperatures on sporulation and disease development could be useful for determination of optimum times for application of fungicides for disease control.

A454

VEGETATIVE COMPATIBILITY AND VIRULENCE OF COLLETOTRICHUM DEMATIUM FROM SPINACH. J. C. Correll, J. C. Guerber, M. Abdelshife, and T. E. Morelock*. Dept. of Plant Pathology and *Dept. of Horticulture and Forestry, University of Arkansas, Fayetteville, AR 72701.

Anthracnose, caused by Colletotrichum dematium, is a destructive disease of spinach in the Arkansas River Valley where over 4,000 acres of spinach are grown annually. Anthracnose can occur as a primary infection or as an infection on white rust lesions caused by Albugo occidentalis (piggy-back infections). Isolates recovered from both primary and piggy-back infections were compared. Sixty-two isolates were examined for both vegetative compatibility, using nitrate non-utilizing mutants (nit mutants), and virulence, using a standardized greenhouse pathogenicity test. Colony morphology, sporulation and spore germination rate were also compared. Two distinct vegetative compatibility groups (VCGs) were identified when Nit1 and NitM mutants, recovered from each isolate, were paired in all combinations. Sixty-two percent (18/29) of isolates in VCG1 were recovered from primary lesions and 64% (21/33) of isolates in VCG2 were recovered from white rust lesions. Significant differences (P = .05) in virulence were observed among isolates within each VCG; virulent and weakly virulent isolates were identified in each VCG and from each infection type (primary and piggy-back). Significant differences (P = .05) in spore germination rate were observed among isolates in VCG2. Colony morphology was considerably more variable among isolates in VCG1; several isolates in VCG1 had apparently lost their ability to produce acervuli.

A455

EFFECT OF SOIL MOISTURE ON THE PRODUCTION OF SPORE-BEARING STRUCTURES AND SPORE RELEASE IN *DIAPORTHE PHASEOLORUM* VAR *CAULIVORA*. K.V. Subba Rao, G.B. Padgett, J.P. Damicone, J.P. Snow, and G.T. Berggren. Dept. of Plant Path. and Crop Phys., LAES, LSU Agric. Center, Baton Rouge, LA 70803.

The influence of soil moisture on the number of pycnidia and perithecia produced, duration of production, and conidial and ascospore release in *Diaporthe phaseolorum* var. *caulivora* (Dpc) was studied. Dpc-infected stem pieces of susceptible soybean 'Bedford' were incubated at 25 C on the surface of soil samples adjusted to matric potentials of 0, -0.1, -0.2, -0.4, -0.8, -1.6, and -3.2 bars. The number of pycnidia and perithecia produced and the number releasing conidia and ascospores, respectively, were counted on 1 cm^2 area of the stem pieces at 15-day intervals. Pycnidia and perithecia were produced after 15 days incubation in 0 to -0.8 bar, while in the drier treatments (-1.6 and -3.2 bars), they were produced after 30 days incubation. Maximum production of pycnidia and perithecia occurred between 30 and 45 days after incubation in 0 to -0.8 bar. Conidia and ascospores were released from the spore-bearing structures in a gelatinous matrix. Spore release occurred only in treatments 0 to -0.8 bar. These observations indicate that Dpc requires different soil moisture regimen for the production of spore-bearing structures and spore release.

A456

THE INCIDENCE AND IMPACT OF DISEASES IN A WILLOW BIOMASS PLANTATION. T.L. Burtless, P.D. Manion, and L.P. Abrahamson. SUNY College of Environmental Science and Forestry, Syracuse, NY 13210.

A monthly disease survey of 275 willow clones in a woodgrass biomass plantation (one year cutting cycle) was conducted to assess disease incidence and its effects on growth. Shoot blight (Colletotrichum gloeosporioides and Pollacia saliciperda) incidence increased during August to 10% and declined in September. Septoria spp. and Marssonina spp. (brown spot) incidence increased throughout the growing season to 20% in September. Melampsora rust incidence increased from 5% to 18% from August to September. Clones with these diseases grew as high or higher than undiseased clones. Cytospora fugax caused localized mortality in one clone. Shoot and foliage pathogens appear to have had minimal annual impact on growth, but long term and intraclonal impacts were not assessed.

A457

SPATIAL ANALYSIS OF VIRULENCE VARIATION IN EUROPEAN POPULATIONS OF *ERYSIPHE GRAMINIS* F.SP. *HORDEI*. J.M. McDermott and E. Limpert. Institut für Pflanzenwissenschaften, Phytomedizin/Pathologie, ETH-Zentrum, CH-8092 Zürich Switzerland.

Powdery mildew of barley is the most important foliar disease of barley in Europe. Considerable effort is being expended in monitoring the air-spora throughout Europe, generating a large spatially-structured data base of variation for virulence and fungicide sensitivity. These data are yielding critical

information on regional differentiation and dynamics of barley mildew populations. Spatial statistical analysis are employed to investigate geographically distributed polymorphisms and to develop *post hoc* explanations for existing genetic variation. This should provide an improved conceptual basis for the analysis of current strategies for disease control and the development of new approaches.

A458

EFFECTS OF IMAZAQUIN, CHLORIMURON ETHYL, AND GLYPHOSATE ON *IN VITRO* GROWTH AND DEVELOPMENT OF *CALONECTRIA CROTALARIAE*. D. K. Berner, G. T. Berggren, and J.P. Snow. Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

Commercial herbicide formulations of imazaquin, chlorimuron ethyl, and glyphosate were evaluated for their effects on *Calonectria crotalariae*, the causal agent of red crown rot of soybean and Cylindrocladium black rot of peanut. Isolates of the fungus from soybean were grown on a medium amended with either water or volumes of the respective herbicides at 0.5, 1, 2, and 4X labeled rates for weed control in soybeans. Glyphosate, in both surfactant and non-surfactant containing formulations, significantly reduced colony area compared with the water control. Simulated repeat applications of imazaquin and chlorimuron ethyl also reduced colony area. Imazaquin at rates of 1X or greater significantly inhibited microsclerotia production. Additions of amino acids to herbicide amended media failed to prevent observed herbicide effects. When used in combination, 0.5X imazaquin and 0.5X glyphosate significantly reduced fungal growth, indicating the potential of these materials as economical and simultaneous preplant weed and disease control agents.

A459

DNA POLYMORPHISM AND PATHOTYPE VARIATION IN THE RICE BLAST FUNGUS. Morris Levy, M. A. Marchetti[1] and J. E. Hamer. Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana and [1]USDA-ARS, Box 999, Beaumont, Texas.

A repetitive DNA family (named MGR sequences) diagnostically marks the genomes of the rice blast pathogen, *Magnaporthe grisea* (*Pyricularia oryzae*). Southern hybridizations with an MGR probe (pCB586) produce genotype-specific DNA "fingerprints" (EcoRI restriction fragment length profiles) among all field isolates tested. A blind-test experiment now shows that these DNA fingerprints distinguish the major pathotypes in the USA (Intl. races IB-1, IB-45, IB-49, IB-54, IC-17, ID-13, IG-1, AND IH-1), reliably index pathotype diversity among USA field isolates collected from 1959-1998, and indicate the phylogenetic relatedness among pathotypes, e.g., IB-49 is composed of two distantly related lineages, one of which shares recent common ancestry with IB-54. These results resolve lingering questions about pathotypic stability in *M. grisea* and illustrate new opportunities for determining population dynamics and evolution of this important crop pathogen.

A460

FOLIAR AND SOILBORNE ASPECTS OF PATHOGENICITY OF COLLETOTRICHUM COCCODES ON RUSSET BURBANK POTATO. A.W. Barkdoll and J.R. Davis, Univ. of Idaho Research and Extension Center, Aberdeen, ID 83210.

Pathogenic variation and symptom expression of C. coccodes on Russet Burbank potato were evaluated with root inoculations in the greenhouse and foliar inoculations in the field and the greenhouse. Potato foliage was treated with air-blown sand (40 Kph air speed) to induce wounding and simulate wind storm damage and sprayed with conidial suspensions of each isolate. Plants were misted to establish infection. In the greenhouse, leaf lesions resembling those produced by Alternaria solani and wilt resembling Verticillium wilt were observed. All isolates reduced tuber yield. Wilt incidence was negatively correlated with tuber yield and specific gravity ($p=0.001$). In the field, one isolate reduced total tuber yield and yield among larger tubers (>280g). Pathogenicity from soil inoculum was studied by mixing ground, colonized oats to produce soil inoculum levels (100-180 cfu/g soil) comparable to those observed in a soil survey of potato production areas. Most isolates significantly reduced root dry weight and some reduced tuber yield. Root cortical damage ranged from slight to extreme.

A461

PHENOTYPIC AND GENOTYPIC MARKERS IN HOST SPECIALIZED ISOLATES OF *SPORISORIUM REILIANUM*. G. Naidoo, R. A. Frederiksen, D. G. Bai, and C. W. Magill, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas 77843-2132.

Both maize and sorghum are hosts of *Sporisorium reilianum*, the causal organism of head smut. Some isolates colonize only maize, while others colonize sorghum predominantly and sudan grass and maize to a lesser extent. In the USA the latter type has been further delineated with the use of a sorghum host differential series. Light and scanning electron microscopy reveal that teliospores from sori on maize and sorghum are morphologically indistinguishable. Differences in optimal conditions for germination and growth were detected. Starch gel-electrophoresis of sporidial extracts did not provide any distinctive isozyme marker. Differences found between maize and sorghum isolates using IEF-PAGE separation were not consistent for all isolates. IEF-PAGE separations and RPLP's are being examined in order to obtain unique isozymic and genetic markers.

A462

PREPARATION OF DRY, VIABLE MYCELIA OF SCLEROTINIA MINOR. H. A. Melouk and C. Bowen, USDA-ARS and Department of Plant Pathology, Oklahoma State University, Stillwater 74078-9947.

Fifty ml of potato-dextrose (PD) broth with streptomycin sulfate (SS), 100 ug/ml, in 250 ml bottles were each inoculated with a plug (15 mm diam) from a 2-day old culture of S. minor grown on PD-agar with SS (SPDA). Bottles were placed on a shaker (15 rpm) at 25 C for five days. Mycelia were collected by centrifugation (2000g), 20 min at 4 C, suspended in 20 ml of 5 to 15% polyethylene glycol (MW 6000), and then collected by centrifugation followed by filtration on a Millipore filter (0.45 um). Filters with mycelia were dried at 25 C for 2 days in a desiccator with $CaSO_4$. Viability of mycelial fragments was determined by plating on SPDA. Dry mycelia were stored at 40 or 80% relative humidity (RH) for up to 10 wk with periodic determination of viability. A significant loss of viability of mycelia occurred after 4 wk storage at 80% RH and continued to increase until the experiment was terminated at 10 wk. Storage of mycelia at 40% RH up to 10 wk did not affect viability.

A463

AFLATOXIN CONTROL IN PREHARVEST CORN (ZEA MAYS): EFFECTS OF CHITOSAN AND TWO MICROBIAL AGENTS. R.G. Cuero, E. Duffus, G. Osuji, and R. Pettit*. Prairie View A&M University, CARC, Prairie View, Texas 77446, and *Texas A&M University, Department of Plant Pathology and Microbiology, College Station, TX 77843.

Aspergillus flavus growth and aflatoxin B production in preharvest corn kernels was determined after treatment with chitosan, Bacillus subitlis, and Trichoderma harzianum. Individual or combined control treatments were applied at the milk stage of ear development, 48 h before or after kernel inoculation with aflatoxigenic A. flavus isolate. Both single and combined treatments reduced A. flavus growth and aflatoxin production, but there was no consistent correlation between funal growth and aflatoxin production. Untreated kernels, inoculated with A. flavus, produced 1104 ppb of aflatoxin B. Toxin accumulation was significantly ($p = < 0.10$) reduced, in same instance to non detectable (ND) levels, by single treatments applied 48 h before A. flavus inoculation inhibited aflatoxin production (ND and 2 ppb respectively).

A464

THE CORRELATION OF RHIZOMOBILITY AND BIOLOGICAL CONTROL POTENTIAL OF RHIZOBACTERIA. I.J. Misaghi and M.W. Olsen. Department of Plant Pathology, University of Arizona, Tucson, AZ 85721

Determination was made of the importance of rhizomobility (a term used here to denote the ability of a bacterium to move along

roots in the absence of percolating water) of rhizobacteria in their ability to control bacterial wilt of tomato caused by Pseudomonas solanacearum. Ten rhizomobile isolates and ten non-rhizomobile isolates of Bacillus sp., P. fluorescens, and P. putida, were compared for rhizomobility and biological control potential. All ten rhizomobile isolates and only two of ten non-rhizomobile isolates provided statistically significant (P=0.05) reduction in disease incidence. Moreover, rhizomobile isolates reduced disease incidence at significantly higher levels than non-rhizomobile ones. All rhizomobile isolates and only five of ten non-rhizomobile isolates colonized tomato roots. These results point out the importance of rhizomobility and root colonizing ability in biological control activity of tested rhizobacteria.

A465

DUAL CULTURES OF POTATO AND A PLANT GROWTH-PROMOTING RHIZOBACTERIUM. M. Frommel[1], S. Ma[1], J. Nowak[2] and G. Lazarovits[1], [1]Ag. Can., Research Ctr., 1400 Western Road, London, Ont., N6G 2V4 and [2]Nova Scotia Agricultural College, Truro, N.S., B2N 5E3.

A nonfluorescent Pseudomonas sp (PSJN222) isolated from onion rhizosphere strongly promoted "in vitro" growth of potato plantlets. On micropropagated plants, the bacteria elicited significant increases in root and haulm dry weight, stem length and adventitious root number when compared to uninoculated controls. Bacterization promoted secondary root branching as well as increased root, stem and leaf hair formation. Total lignin content of bacterized plantlets was significantly augmented. The mechanisms of growth enhancement are not yet known but the involvement of a 90 Kb megaplasmid is being investigated. Various mutagens were used in order to produce a non-growth promoting mutant, and to eliminate the plasmid from PSJN222. The effects of bacterization on field survival, performance and yield of micropropagated potato plantlets was also studied. In order to facilitate the identification of the bacteria in colonization studies, a recombinant plasmid encoding luciferase activity was constructed and incorporated into PSJN222.

A466

THE USE OF SURFACTANTS TO FACILITATE INFECTION OF LEAVES BY BACTERIAL PLANT PATHOGENS: IMPLICATIONS FOR BIOLOGICAL WEED CONTROL. N.K. Zidack and P.A. Backman. Dept. of Plant Pathology, Auburn University, Auburn, Al. 36849.

Bacteria have been largely ignored as potential bioherbicides due to their sensitivity to environmental effects and their inability to directly penetrate plant tissue. Their application has been limited due to requirements for wounds, vectors and/or free water for the invasion of nectaries, stomata and hydathodes. Reports indicate that surfactants capable of reducing the aqueous surface tension below 25mN m^{-1} can cause wetting of substomatal cavities. A stomate-flooding surfactant has been shown to greatly enhance infection when sprayed with plant pathogenic bacteria. Pseudomonas syringae pv. phaseolicola (P.s.p.), a pathogen of kudzu (Pueraria lobata) and common bean (Phaseolus vulgaris) was applied to greenhouse grown bean plants with and without surfactant. When applied with surfactant, high levels of disease developed and infection was independent of leaf surface moisture. Without surfactant, no disease developed on inoculated plants.

A467

DETECTION OF GLIOTOXIN PRODUCED IN SITU BY GLIOCLADIUM VIRENS IN THE BIOCONTROL OF PYTHIUM AND RHIZOCTONIA DAMPING-OFF OF ZINNIA. R. D. Lumsden, J. C. Locke, and S. T. Adkins, USDA, ARS, Beltsville, Maryland 20705.

Gliocladium virens (GL-21) produced the antibiotic, gliotoxin, in soilless mix (0.42 µg/cc), composted soil (0.36 µg/cc), loamy clay soil (0.20 µg/cc), and sandy loam (0.02 µg/cc), when added (0.1% w/v) as a bran-alginate formulation (W. R. Grace & Co.-Conn.). Gliotoxin (extracted with chloroform and detected by HPLC) quadrupled when 0.4% (w/v) prill was added. Growth in vitro of P. ultimum was completely inhibited when extracts contained at least 1 µg gliotoxin/cc of soil media. Biocontrol of damping-off of zinnia caused by P. ultimum and R. solani was effective with both of the above amounts of GL-21 prill. Gliotoxin was detected in soilless mix 2 and 7 days after amendment with prill, but not at zero time or after 14 days. Aqueous extracts from Gliocladium-amended soilless mix was effective in reducing damping-off caused by Pythium. These results indicate a role of gliotoxin in biocontrol.

A468

REDUCTION OF DETRIMENTAL EFFECTS OF APHID HONEYDEW BY YEASTS ON WHEAT LEAVES. A.J.Dik, N.J.Fokkema and R.Rabbinge. Willie Commelin Scholten Phytopathological Laboratory, Baarn, the Netherlands, Research Institute for Plant Protection (I.P.O.), Wageningen, the Netherlands, Department of Theoretical Production Ecology, Agricultural University, Wageningen, the Netherlands.

Aphid honeydew on wheat leaves causes yield losses by interfering with the physiology of the leaves and stimulating necrotrophic pathogens. Naturally occurring saprophytic yeasts may use honeydew as a nutrient source. However, the yeasts are often reduced by broad-spectrum fungicides added to selective chemicals. In field experiments yeasts effectively removed aphid honeydew, while accumulation of honeydew occurred when the yeast population was reduced by maneb. Furthermore, both in a controlled environment and in the field the effectivity of selective fungicides was reduced by honeydew. Protection of yeasts by only spraying with selective fungicides can reduce aphid damage and enhance fungicide effectivity by reduction of accumulation of honeydew on the leaves. A simulation model for honeydew consumption by yeasts was developed.

A470

MUTATIONS IN PSEUDOMONAS FLUORESCENS IMPROVE ANTIBIOSIS AND BIOCONTROL OF TAKE-ALL OF WHEAT. Yufa Peng and A. H. Ellingboe. Chinese Academy of Agricultural Sciences, Beijing, People's Republic of China, and University of Wisconsin-Madison, WI 53706.

Tn5 induced mutants in Pseudomonas fluorescens were found that have altered abilities to express antibiosis against Gaeumannomyces graminis. Four of seven mutants with increased antibiosis consistently gave control of the take-all disease of wheat in greenhouse experiments. Wild type strain CN12 and a mutant, D93, derived from CN12 with increased antibiosis were tested as seed treatments on spring wheat in Ningxia Autonomous region of the People's Republic of China. Tests were conducted in three separate fields with three replicates in each location. Seed treatment with strain CN12 increased yield 13% compared to the control of no seed treatment. Strain D93 increased yield 28% over no treatment. The 15% higher yield with D93 compared to CN12 represented a highly significant increase in yield.

A471

BIOLOGICAL CONTROL OF COTTON SEEDLING DAMPING-OFF BY COATING COTTONSEED WITH GLIOCLADIUM VIRENS PREPARATIONS. C. R. Howell, USDA-ARS Southern Crops Research Lab, Rt. 5, Box 805, College Station, Texas 77840.

Biocontrol of Pythium or Rhizoctonia induced cotton seedling damping-off depends on the biocontrol strain, the substrate it is grown on, and the amount of inoculum applied. Optimum biocontrol was obtained by air drying 6-day old G. virens-substrate combinations, grinding them to 500 micron particle size, and coating them on seed with latex sticker (one gram per 100 seed). Failed strain-substrate combinations produced little antifungal antibiotic, too much of the herbicide viridiol, or lost viability too quickly to effect disease control. The best strain-substrate combination (GV-P on millet) remained biocontrol effective for 3 months, and viable for 5 months. Biocontrol activity could be extended by priming the preparation with 100% relative humidity 24 hrs. before use. Loss of biocontrol efficacy was associated with a significant lengthening of propagule germination time. Optimum germination time was 24 hrs. or less.

A472

BIOCONTROL OF WHITE MOLD DISEASE OF DRY BEAN WITH ERWINIA HERBICOLA. G.Y. Yuen, G. Godoy, and J.R. Steadman. Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583

White mold disease (Sclerotinia sclerotiorum) was reduced in dry bean by an epiphytic strain B1 of Erwinia herbicola. This strain and Bacillus polymyxa B8, both isolated from bean blossoms and found to inhibit ascospore germination and mycelial growth of the pathogen in vitro, were tested in a field experiment for control of the disease in comparison to a single spray application of benomyl. Following application of cell suspensions onto plants, populations levels of the test strains

were monitored using semi-selective and differential media. Population levels of B1 detected on blossoms of B1-treated plants exceeded log 4 colony-forming units over 14 days. Treatment with strain B1 resulted in disease severity which was significantly lower than that of the nontreated control and similar to that with benomyl. In contrast, B8 did not establish on blossoms in appreciable numbers and also failed to inhibit white mold. None of the treatments provided any improvement in yield over the control.

A476

THE DISTRIBUTION OF ANTAGONISTIC BACTERIA BY HONEY BEES FOR BIOLOGICAL CONTROL OF FIRE BLIGHT. S. V. Thomson and K.M. Shotwell (Dept. of Biology), J.D. Vandenberg (USDA-ARS Bee Lab). Utah State Univ., Logan, UT 84322-5305.

Biological control of fire blight can be achieved by protecting pear and apple flowers with antagonistic bacteria. However, orchard spraying may not be the most efficient application method. Honey bees were used to deliver apple pollen treated with a suspension of antagonistic bacteria (10^8 cfu/ml). The bees successfully delivered bacteria to newly opened flowers. The bacteria subsequently multiplied on the flowers and survived for at least 2 weeks. Biological control of fire blight is dependent on the antagonist used, the concentration of bacteria deposited on the stigma, the environmental conditions and the inoculum level of Erwinia amylovora.

A477

SEQUENCE ANALYSIS OF THE SWEETPOTATO FEATHERY MOTTLE VIRUS COAT PROTEIN GENE REVEALS A 509 bp CLONE NEAR THE 3' END THAT IS HIGHLY HOMOLOGOUS TO ALL KNOWN STRAINS. J. A. Abad and J. W. Moyer. Dept. of Plant Pathology, N.C. State Univ., Raleigh, NC 27695-7616.

Subcloning of 500 bp and 1400 bp Hinc II fragments derived from a 2000 bp cDNA of SPFMV (strain RC) containing the coat protein gene in vitro transcription vector plasmid pGem 4Z (Promega) and pGem 3Zf+ (Promega) generated plasmids pG4FMRC4.05 and pG3FMRC4.14 respectively. These plasmids served as templates for phage T-7 RNA polymeraze to synthesize 32-p labeled cRNA probes (Riboprobes). Slot-blot hybridization assays under high stringency conditions showed that pG4FMRC4.05 Riboprobe detected all known strains of SPFMV (RC, YV, 835, and C). In contrast, pG3FMRC4.14 did not detect strain C which is distantly related serologically. Sequence analysis of these cDNAs revealed that pG4FMRC4.05 contains 509 bp cDNA near the 3' end of the gene, whereas pG3FMRC4.14 encodes for the predicted putative amino acid sequence for proteolytic cleavage typical of Potyviruses. The complete analysis of the coat protein gene is in progess.

A478

SYMPTOM DETERMINANTS AND PHENOTYPE OF THE VIRAL RNA AND AN INFECTIOUS cDNA CLONE OF A MASKED STRAIN OF TMV. R.S. Nelson[1], C. Holt[2], R.A.J. Hodgson[2], F.A. Coker[1] and R.N. Beachy[2], [1]Noble Foundation, P.O. Box 2180, Ardmore, OK 73402, [2]Washington University, Box 1137, St. Louis, MO 63130.

An infectious cDNA clone of a strain of tobacco mosaic virus that produces attenuated systemic symptoms on Nicotiana tabacum cv. Xanthi has been constructed. Through fragment swapping experiments, where portions of a cDNA clone from a green mosaic producing strain have been switched with portions of the attenuated cDNA sequence, the cause of the attenuation has been mapped to the ORF encoding the 126kD protein. Sequence analysis shows multiple base changes that would result in amino acid changes. Since the attenuated strain produces normal size necrotic lesions and normal virus accumulation in inoculated leaves, virus replication and spread in inoculated and systemically infected leaves over time will be analyzed by tissue and RNA blots. These results will help determine how the base changes in the attenuated strain result in the masked phenotype.

A479

A POINT MUTATION WITHIN A CMV SATELLITE RNA ALTERS THE HOST SPECIFICITY OF CHLOROSIS INDUCTION. D. E. Sleat and P. Palukaitis, Dept. of Plant Path., Cornell University, Ithaca, NY 14853.

Various cucumber mosaic virus (CMV) satellite (sat) RNAs can induce chlorosis on either tobacco or tomato plants. On tobacco, B2- and WL3-sat RNAs only induced chlorosis with subgroup II CMV helper strains, whereas no helper specificity was observed with B3-sat RNA on tomato. Sequence comparisons of a number of chlorosis-inducing satellite RNAs indicated a single nucleotide position that might dictate whether a given satellite RNA could induce chlorosis on tomato or tobacco. This position was mutated in a cDNA clone of a sat RNA which only induced chlorosis on tomato, and the resulting transcripts were inoculated onto tomato and tobacco. The mutant satellite RNA no longer induced chlorosis on tomato, but instead, induced chlorosis on tobacco that was phenotypically identical to that induced by B2- and WL3-sat RNAs. These results indicate that a single domain is responsible for chlorosis induction on both tomato and tobacco, but that the specificity is controlled by a single nucleotide. The induction of chlorosis on tobacco and tomato plants by CMV satellite RNAs therefore appear to be mutually exclusive pathogenic effects.

A480

MOLECULAR CLONING AND CHARACTERIZATION OF CLONES OF TOMATO SPOTTED WILT VIRUS. M. Wang, J. S. Hu, and D. Gonsalves, Department of Plant Pathology, Cornell University, NYSAES, Geneva, NY 14456

Tomato spotted wilt virus (TSWV) is the sole member of the TSWV group. The virus has a single strand RNA genome consisting of three RNA molecules of 3.4 Kb (RNA-S), 5.2 Kb (RNA-M), and 8.3 Kb (RNA-L). The complementary DNA was synthesized by random priming to the RNAs of the BL isolate of TSWV purified from *Datura stramonium* and cloned into plasmid vector pUC18. Several clones were found containing cDNA specific to RNA-M of the virus after Northern blot hybridization. The largest size of the inserts is about 1.6 Kb. A full length clone (3.4 Kb) of RNA-S was identified by the same type of assay. These TSWV clones do not react to healthy plant materials when tested by Northern blot hybridization with purified *D. stramonium* nucleic acid or by dot blot hybridization with crude or purified *D. stramonium* extract. The sequences of these clones are being determined.

A481

Immunological detection of the red clover necrotic mosaic virus polymerase expressed by ribosomal frameshifting. Z. Xiong, T. L. Kendall, and S. A. Lommel, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC, 27695-7616.

Sequence data suggests that the red clover necrotic mosaic virus (RCNMV) RNA-1 encoded replicase is expressed and regulated by a ribosomal frameshift mechanism which is structurally and functionally identical to the retroviral *pol* gene frameshift element. Two distinct open reading frames (ORFs) are identified in RNA-1 in addition to the 3' terminal capsid protein ORF. A pre-frameshift 27 kDa protein and a lesser amount of an 88 kDa protein (p88) are observed. p88 arises from ribosomal frameshifting at the end of the first ORF with continued translation through the second ORF. Oligopeptides were synthesized to an internal region of the post-frameshift, and the C-terminus of p88. Oligo-peptides were conjugated to KLH and an antiserum raised in rabbits. Oligo-peptide specific antibodies were concentrated on an oligo-peptide affinity column. The polymerase antibodies immunoprecipitated p88 but not the pre-readthrough p27 translation products. In addition, a viral encoded 57 kDa product was also recognized by the antibody. These data suggests that the p88 is expressed at catalytic levels controlled by the frameshift element.

A482

TRANSGENIC NICOTIANA BIGELOVII THAT EXPRESS THE GENE VI PRODUCT OF CAULIFLOWER MOSAIC VIRUS (CAMV) COMPLEMENT A CAMV STRAIN THAT IS DEFECTIVE IN SYSTEMIC SPREAD. J.E. Schoelz, *K.-B. Goldberg, and *J.M. Kiernan. Dept. of Plant Pathology, University of Missouri, Columbia, MO and *Dept. of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Gene VI of CaMV recombinant virus H12 was transfered into the genome of N. bigelovii through the use of the Agrobacterium vector pGA472. Virus H12 was chosen as the source of gene VI because previous work demonstrated that the gene VI product of this virus determined systemic infection of N. bigelovii. In order to demonstrate complementation, transgenic plants that expressed the CaMV gene VI product and nontransformed controls were inoculated with recombinant virus H31, a virus which could not systemically infect non-transformed N. bigelovii. Transgenic N. bigelovii inoculated with H31 developed systemic symptoms 3-4 weeks after inoculation, while the nontransformed N. bigelovii controls remained symptomless. In order to show that no changes had occured within the H31 genome that would affect host specificity, the H31 virus was isolated from systemically infected leaves of the transgenic plants and re-inoculated to both nontransformed and transgenic N. bigelovii. Again, the transgenic N. bigelovii developed systemic symptoms while the nontransformed controls remained healthy.

A483

NUCLEOTIDE SEQUENCE OF SOIL-BORNE WHEAT MOSAIC VIRUS RNA II. Y. Shirako, I. Ali, and T. M. A. Wilson. AgBiotech Center, Rutgers Univ., New Brunswick, NJ 08903-0231.

Nucleotide sequences of RNA II of wild type and a deletion mutant of soil-borne wheat mosaic virus were determined from cloned cDNA. Next to the 5' untranslated region (ca. 330 nt long), there is an open reading frame (ORF) for a 19.3 K polypeptide terminated with an opal codon, followed by a continuous, in-frame coding region for an additional 64.4 K (= 83.7 K polypeptide) stopped by an ochre codon. From *in vitro* translation results, the 19.3 K polypeptide corresponds to the capsid protein and the 64.4K portion is expressed as a read-through product of the capsid protein. Towards the the 3' end, there is another ORF for a 18.8 K polypeptide, followed by the 3' untranslated region (ca. 400 nt long). Mutant RNA II has two internal deletions in the read-through (64.4 K) region; 108 nt immediately after the capsid protein gene and 1058 nt before the ochre termination codon. Apart from these deletions, 99.5 % of the nucleotide sequences and the genome organization of the two isolates were identical.

A484

COMPARISON OF THE 3' TERMINAL SEQUENCES OF TOMATO SPOTTED WILT VIRUS ISOLATES. M. D. Law and J. W. Moyer, Department of Plant Pathology, Raleigh, NC 27695-7616.

A tomato spotted wilt-like virus (TSWV-I) isolate with a serologically distinct N protein has been identified which predominantly infects ornamental crops. Complementary DNA (cDNA) was synthesized to both TSWV and TSWV-I by artificially polyadenylating genomic RNA. cDNA probes specific for either TSWV-I S RNA or TSWV-I M RNA did not cross-hybridize with TSWV RNA's. TSWV has been shown to have a conserved 8 nucleotide sequence on the 3' end of both the S and M RNA. Sequence analysis of the TSWV S cDNA confirmed the TSWV consensus sequence in our TSWV isolate (common serotype). The 3' terminal RNA sequence of TSWV-I S and M RNA was determined from the cDNA clones and compared to the published TSWV 3' terminal consensus sequence. The TSWV-I M RNA 3' terminal sequence was identical to the TSWV consensus sequence. In contrast, the TSWV-I S 3' terminal sequence did not contain the entire TSWV consensus sequence.

A485

USE OF CHIMERIC COAT PROTEIN CONSTRUCTS AND DELETION MUTANTS TO EXAMINE POTYVIRUS STRUCTURE AND COAT PROTEIN MEDIATED RESISTANCE. J. Hammond, R. L. Jordan and K. K. Kamo, USDA-ARS, Beltsville, MD 20705.

Several monoclonal antibodies (MAbs) prepared to potyviruses react with a fusion protein expressed in E. coli from a cDNA clone containing the complete coat protein (CP) gene of bean yellow mosaic virus (BYMV). Some of the MAbs also react with CPs of other potyviruses. Chimeric CP genes were constructed between BYMV and pepper mottle virus or zucchini yellow mosaic virus. Carboxyterminal BYMV CP deletion mutants were provided termination codons from an oligonucleotide. The chimeric and truncated constructs expressed in E. coli were analyzed by Western blotting. MAb reactivity was compared to amino acid sequences of the CPs. Constructs are being expressed in Nicotiana benthamiana to examine the contribution of different domains to the protective effect reported in plants expressing viral CP.

A486

PRODUCTION AND INITIAL CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO HELPER COMPONENTS OF POTATO VIRUS Y AND TOBACCO VEIN MOTTLING VIRUS. Ramon Jordan, David Thornbury and Tom Pirone, USDA-ARS, Florist & Nursery Crops Lab., Beltsville, MD and Dept. of Plant Pathology, Univ. of Kentucky, Lexington, KY.

Helper component (HC) preparations purified from potato virus Y (PVY)- and tobacco vein mottling virus (TVMV)-infected plants by oligo (dT)-cellulose chromatography were used to generate a panel of monoclonal antibodies (McAbs). HC-specific McAb-secreting hybridomas (102) were selected using sucrose gradient purified HC preparations in antigen-coated plate and triple-antibody-sandwich ELISAs. Fifty-one McAbs were specific to TVMV-HC, 25 were specific to PVY-HC and the remaining 26 reacted with both TVMV- and PVY-HC. Some of the TVMV-HC-, PVY-HC-specific and cross-reactive McAbs also reacted with crude sap preparations of bean yellow mosaic virus, pepper mottle virus, and/or aphid and non-aphid transmissable strains of tobacco etch virus. These McAbs may be useful probes in experiments designed to examine the role of specific HC protein sites in HC-mediated aphid transmission of potyviruses.

A487

DNA SEQUENCE OF PEANUT CHLOROTIC STREAK VIRUS. R. D. Richins, T. Broos, S. G. Gowda, D. A. Ducasse, D. V. R. Reddy, and R. J. Shepherd. Dept. of Plant Pathology, Univ. of Kentucky, Lexington, KY 40546.

Peanut chlorotic streak virus (PClSV) is a caulimovirus which infects peanuts (*Arachis hypogaea)* as well as a variety of solanaceous plants of India. An infectious clone, pPClSV-K1, was prepared by ligating *Kpn* I digested PClSV DNA into pUC119. Subclones of pPClSV-K1 were sequenced by the Sanger dideoxynucleotide method. The 8.2 kilobase genome is organized into eight open reading frames (ORFs). Based on size, position, and homology, seven of these putative genes appear to correspond to similar genes of other caulimoviruses. However, the degree of relatedness of the inferred amino acid sequences is considerably less than that between similar polypeptides in other caulimoviruses. In addition, a novel ORF, occurring between ORFs II and III is observed in the PClSV genome. Like most other caulimoviruses, PClSV's genome contains a large intergenic region between ORFs VI and VII. However, the small intergenic region which occurs between ORFs V and VI in the genomes of cauliflower mosaic virus, figwort mosaic virus and carnation etched ring virus is not present in the genome of PClSV.

A488

DIRECT RNA SEQUENCING FOR IDENTIFICATION AND DETERMINATION OF GENETIC RELATEDNESS OF POTYVIRUSES INFECTING WHEAT N. L. Robertson, R. French, and W. G. Langenberg. USDA-ARS, Department of Plant Pathology, University of Nebraska, Lincoln NE 68583.

The genetic relatedness among four isolates of wheat streak mosaic virus (WSMV), hordeum mosaic virus (HMV), and agropyron mosaic virus (AMV) infecting wheat was compared by direct RNA sequencing. Like definitive potyviruses, these cereal viruses contain poly(A) tails. Synthetic $dT_{14}A$, $dT_{14}C$, or $dT_{14}G$ primers specifically initiated

cDNA synthesis on polyadenylated RNAs; other than the presence of a poly(A) tail, no prior sequence information is necessary. At least 200 3'-terminal bases were determined for WSMV and HMV. Comparison of the profiles showed that four WSMV isolates ('Wyoming', 'Sidney', 'Type', and 'Corn') were over 96% identical, while no sequence similarity existed among WSMV, HMV, and AMV. Determination of the easily accessible 3'-terminal sequence is a rapid procedure for precise identification of distinct viruses and their strains, and is a practical alternative to time consuming plant host range and vector tests, serological and electron microscopy methods, and molecular cloning for many purposes. Non-polyadenylated RNAs can also be sequenced by this method after "tailing" with poly A polymerase.

A489

DELETION ANALYSIS OF BROMOVIRUS 2a PROTEIN: EFFECTS ON RNA REPLICATION AND SYSTEMIC SPREAD

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Brome mosaic virus (BMV) 1a and 2a proteins are required for viral RNA replication. The central region of 2a contains a polymerase-like domain, and shows extensive similarity with nonstructural proteins in other viruses, such as nsP4 in Sindbis and the 183 kD protein in tobacco mosaic virus (TMV). BMV 2a protein contains additional amino- and carboxy-terminal domains which have no counterparts in the TMV and Sindbis proteins. Frame-shift and deletion mutants of BMV RNA2 were used to investigate the role of these flanking domains in directing RNA replication in protoplasts, and possible involvement of RNA2 or 2a protein sequences in systemic spread of BMV in barley.

Results with altered 2a proteins show that the C-terminal 125 residues are dispensable for RNA replication. N-terminal deletions of 22 and 49 amino acids produced 2a proteins with progressively less activity relative to wildtype; larger deletions were lethal. Both N- and C-terminal deletion mutants supported systemic infection of barley plants, although the yield of virus was substantially reduced compared to wildtype infection, and did not parallel the relative levels of RNA replication activity in protoplasts. Additionally, symptoms typical of BMV infection were greatly reduced or absent in mutant-infected plants.

A490

CHARACTERIZATION OF AN INFECTIOUS TWO-BASE DELETION MUTANT OF POTATO SPINDLE TUBER VIROID (PSTV). D.K. Lakshman and S.M. Tavantzis, Dept. of Botany and Plant Pathology, Univ. of Maine, Orono, ME 04469.

A two-base deletion mutant (ST4) of PSTV cDNA (bases 339 and 340) was infectious when tandem DNA or (+)RNA dimers were used to inoculate tomato cv. Rutgers. Symptoms resulting from infection with ST4 DNA or (+)RNA dimers appeared later and were considerably milder than those induced by the parental severe strain (SC) of PSTV. Total RNA from ST4- or SC-infected tomato plants was hybridized to radiolabeled *in vitro* transcribed SC (-)RNA, treated with ribonuclease and resolved in an 8% polyacrylamide 7M urea gel. Two bands of 251 and 108 bases, but only one band of 359 bases were found to be associated with extracts of ST4- and SC-infected plants, respectively. These results suggest that ST4 RNA progeny possess the two-base deletion. Sequencing of these progeny is currently underway. The effect of host-passages on the stability of the mutant is under investigation.

A491

TOXICITY AND TOXIN PRODUCTION OF Fusarium ISOLATES FROM SAMPLES COLLECTED IN ALASKA. Weiping Xie, Chester J. Mirocha, Xiaoling Wang, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA; Jennifer McBeath, Department of Plant Pathology, University of Alaska, Fairbanks, AK 99775, USA.

One hundred and seventeen Fusarium isolates were obtained from 84 samples (soil, oat and barley stems) in Alaska in 1986 and 1987. Among 91 isolates checked for toxicity in rats by feeding test, 67 were toxic. Rice cultures of the toxic isolates caused hematuria, diarrhea, hemorrhage in intestines and enlarged uterus in the rats. Fifty-eight isolates produced fusarochromanone (TDP-1) in rice cultures with mean concentration of 1631 μg/g and range from 82 to 3760 μg/g. All the fusarochromanone-producing isolates were identified as F. equiseti. Six isolates produced wortmanin (H1) and were identified as F. sambucinum. This was the first time that wortmanin-producing Fusarium isolates were found in North America. It is most likely that fusarochromanone accounted for the toxicity of F. equiseti isolates and wortmanin for the toxicity of F. sambucinum. Other toxins found included zearalenone and small amounts of trichothecenes.

A492

BIOSYNTHESIS OF TWO FATTY ACID DERIVATIVES OF FUSAROCHROMANONE BY Fusarium equiseti (ALASKA 2-2). Weiping Xie, Chester J. Mirocha, Yechun Wen, Won Jo Cheong and Robert J. Pawlosky, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA.

Two fluorescent compounds with trivial names TDP-9a and TDP-9b were isolated from rice cultures of Fusarium equiseti (Alaska 2-2). Analysis by mass spectrometry indicated that TDP-9a had a molecular weight of 596 with a molecular formula of $C_{35}H_{52}N_2O_6$ and that TDP-9b (molecular weight 598) had a molecular formula of $C_{35}H_{54}N_2O_6$. Hydrolysis of a mixture of the two compounds with 2N HCl in 80% methanol yielded four compounds. They were identified by mass spectrometry as fusarochromanone (TDP-1), 3'-N-acetyl-fusarochromanone (TDP-2), octadecadienoic acid methyl ester and octadecenoic acid methyl ester. The latter two compounds were resolved by gas chromatography with a capillary column. TDP-9a was identified as 3'-N-acetyl-4'-O-octadecadienoic acyl fusarochromanone, and TDP-9b was identified as 3'-N-acetyl-4'-O-octadecenoic acyl fusarochromanone. The locations of the double bonds in the fatty acyl moieties of these two compounds were not identified.

A493

MOISTURE VARIABILITY OF INDIVIDUAL SEEDS OF SOYBEANS AND ITS ROLE ON STORAGE FUNGI DEVELOPMENT. F.A. Lazzari and R.A. Meronuck, Dept. of Plant Pathology, University of Minnesota, St. Paul 55108.

The moisture content (MC) of individual seeds and percentage (%) of seeds infected with storage fungi were determined from six soybean samples stored two years at 75% RH and 25 C. The MC was determined by oven-drying 16 single seeds at 110 C for 20 hours. The % of seeds infected with storage fungi was obtained by plating 60 soybean seeds on T-6 media.

Average MC of the six samples was 11.7, 12.0, 12.2, 12.4, 12.5, and 13.0%. The % of seeds in the samples infected with storage fungi was 0.0, 0.0, 0.0, 40.0, 47.0, and 46.0% respectively. The % of individual seeds with MC above 12.0% (level safe for storage) in the above samples was 12.0, 37.0, 68.0, 100.0, 100.0, and 100.0% respectively.

There was correlation between the % of individual seeds with MC above 12.0% and the % of seeds infected with storage fungi from each sample. Knowing the % of individual seeds with MC over 12% in a given soybean bulk may be an indirect method of measuring the risk of future storage.

A494

PRODUCTION OF ZEARALENONE SULFATE BY Fusarium spp. Javier Plasencia and C. J. Mirocha, Dept. of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

A water-soluble compound related to zearalenone was isolated from a culture of Fusarium graminearum #30 grown in rice. Negative-Ionization Fast-Atom Bombardment mass spectrometry of the metabolite gave a molecular ion with m/z ratio of 397 which is coincident with the mass of the conjugate zearalenone sulfate. Acid hydrolysis yielded zearalenone and a water soluble compound which was reacted with barium chloride precipitating into presumably barium sulfate. The presence of the metabolite in several Fusarium cultures grown in rice was determined using thin layer chromatography. F. graminearum, F. equiseti and F. sambucinum were found to produce it. In the rat uterine-enlargement bioassay, the metabolite was found to have the estrogenic activity characteristic of zearalenone. Natural occurrence of this previously undescribed conjugate might be significant because analytical methods devised for zearalenone in grain cannot detect the conjugate but it retains the biological properties of the mycotoxin when ingested by animals.

A495

A PRELIMINARY STUDY OF POSSIBLE MECHANISMS OF ULTRAVIOLET INDUCED RESISTANCE TO POSTHARVEST ROTS. C. Stevens, J. Y. Lu, V. A. Khan, C. L. Wilson, M. K. Kabwe and H. Zhonk. Dept. of Agricultural Sciences, Tuskegee University, AL 36088 and USDA/ARS/NAA Appalachian Fruit Research Station, Kearneysville, WV. 25430.

Resistance to fruit ripening (increase in firmness, ascorbic acid and decrease in soluble solids) was observed during increased postharvest rot resistance of peaches, apples and tangerines after the application of hormetic doses of ultraviolet irradiation (254 nm UV-C) 20, 30 and 18 days respectively. The increased firmness and low soluble solids of Elberta peaches were associated with UV-C induced resistance to brown rot (Monilinia fructicola). Higher ascorbic acid content of UV-C irradiated Golden Delicious apples and Dancy tangerines was inversely correlated with bacteria (Erwinia spp.) and green mold (Penicillium digitatum) resistance, respectively. Exposure of Jewel sweetpotato, apples and tangerines to certain hormetic UV-C level promoted increases in phenylalanine ammonia lyase (PAL) activity. A close association between increases in PAL and increase in resistance of sweetpotato Fusarium storage rot (Fusarium solani) was observed.

A496

ACTIVITY OF TOXINS FROM CERCOSPORA SOJINA AGAINST SOYBEAN FUNGAL PATHOGENS AND EMBRYONATED EGGS, Rama K. Velicheti and J. B. Sinclair, Dept. of Plant Pathology, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801-4709.

Three chromatographic fractions were recovered from Cercospora sojina, cause of frogeye leafspot of soybeans. A deep red frac-

tion was identified as cercosporin, and a yellow fraction as a mixture of fatty acids. A red fraction was not studied. The radial growth of the following soybean fungal pathogens was inhibited by filter paper discs with 5.0ug cercosporin: Alternaria alternata, Fusarium sp., Macrophomina phaseolina, Phomopsis sojae, and P. longicolla, but not of Cercospora beticola, C. kikuchii, C. nicotiana, C. sojina, Colletotrichum truncatum, or Rhizoctonia solani when incubated under 24 hr light. Cercosporin showed no inhibition of any fungus under continuous dark. A LD_{50} of 50.0ug cercosporin was recorded with embryonated chicken eggs incubated under continuous light. The yellow fraction did not inhibit growth of any fungus under continuous light or dark, nor was toxic to embryonated eggs.

A497

POPULATION DYNAMICS OF *CRYPTOCOCCUS LAURENTII* IN WOUNDS IN APPLE AND PEAR FRUIT STORED UNDER AMBIENT OR CONTROLLED ATMOSPHERIC CONDITIONS. P. A. Shefelbine and R. G. Roberts. USDA-ARS, Tree Fruit Research Laboratory, Wenatchee, WA 98801.

Ripe 'Golden Delicious' apples and 'Anjou' pears were each wounded twice, then 10 µl of a buffered, washed cell suspension of *C. laurentii* (RR87-108) at 5 x 10^6 cfu/ml were placed in each wound. Fruit was stored at 1-2 C in a controlled atmosphere (CA; CO_2=2.0%; O_2=1.5%). Four replicates of apple and pear fruits were sampled 3 times weekly for 20 days for population determinations. *C. laurentii* colonized wounds in both apples and pears, reaching a maximum by day 10 in pears (5.7 x 10^6 cfu/wound) and by day 5 in apples (1.8 x 10^6 cfu/wound). Populations remained constant after reaching a maximum and were significantly ($P<0.05$) greater in pears. In a subsequent experiment populations (10µl of a 5 x 10^7 cfu/ml suspension in each wound) were followed for 60 days in apples (non-ripe) stored under CA or ambient atmospheric conditions. Populations reached a maximum (4.3 x 10^6 cfu/wound) by day 4 under both storage conditions and remained constant. These results establish the microaerophilic nature of *C. laurentii*. Because *C. laurentii* exhibits biological control of fungal postharvest pathogens under ambient atmospheric conditions, it should be equally as effective under the cold temperature, low-oxygen conditions typical of commercial storage.

A498

EFFECT OF ATOXIGENIC STRAINS OF ASPERGILLUS FLAVUS ON PREHARVEST AND POSTHARVEST AFLATOXIN CONTAMINATION OF MAIZE KERNELS. R. L. Brown, P. J. Cotty, and T. E. Cleveland. USDA, ARS, Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA 70124.

Field inoculations of developing maize ear kernels with an aflatoxin-producing strain of Aspergillus flavus produced high levels of aflatoxin at maturity. Simultaneous inoculation with a naturally occurring atoxigenic and a toxigenic strain reduced toxin levels by 76% at maturity when compared with ears inoculated with the toxigenic strain alone. Inoculations done with the atoxigenic strain one day prior to inoculation with the toxigenic strain reduced toxin levels by 95%. The atoxigenic strain also reduced postharvest aflatoxin contamination by both the applied toxigenic strain (by percentages similar to those in preharvest tests) and strains resident on the kernels when inoculated onto kernels after harvest. These results indicate that atoxigenic strains of Aspergillus flavus have potential use as biological control agents of both preharvest and postharvest aflatoxin contamination of corn.

A499

SURVIVAL OF ASPERGILLUS FLAVUS IN CORN DEBRIS AND SOIL FROM IOWA CORN FIELDS. L. E. Sweets, N. K. Baker, J. F. Shearer and L. H. Tiffany. Iowa State University, Ames, IA 50011.

Samples of soil and of stalk and cob debris were collected from 40 corn fields in eight Iowa counties following the 1988 harvest. Additional samples were collected from these fields in the spring 1989, fall 1989 and spring 1990. Soil samples were tested for the presence of Aspergillus flavus by sprinkling approximately 0.5 gm of soil onto the surface of M3S10B agar plates. Pieces of cob and stalk pith approximately 2.0 cm in diameter were pulled out of freshly broken cob and stalk samples and plated on M3S10B agar plates. Plates were incubated at 37°C for 3 days and visually examined for presence of A. flavus colonies. Aspergillus flavus was detected in soil samples from all fields at the end of the first three sampling times. About 70% of the cob pieces from both fall 1988 and spring 1989 samplings were positive for A. flavus. However, with the stalk pieces 42% of the fall 1988 samples and 84% of the spring 1989 samples were positive for A. flavus.

A500

THE BIOCONTROL ACTIVITY OF A YEAST STRAIN US-7 AGAINST POSTHARVEST DISEASES OF FRUITS AND VEGETABLES: POSSIBLE MODE OF ACTION. S. Droby*, E. Chalutz*, and C. L. Wilson**. * ARO, The Volcani Center, Bet-Dagan 50250, Israel; and ** Appalachian Fruit Research Station, USDA, ARS, Kearneysville, WV, USA.

The yeast strain US-7 was found an effective antagonist of several postharvest diseases of fruits and vegetables. It effectively inhibited the development of decay caused by Penicillium digitatum, P. italicum and Geotrichum candidum on citrus fruit, and Rhizopus stolonifer and Botrytis cinerea on apples, peaches and grapes.
Studies on the mode of action of US-7 revealed that it does not inhibit the pathogen through production of antibiotic substances. Other possible modes of action investigated were competition with the pathogen for nutrients and/or space, induction of the host resistance mechanisms and direct interaction between the antagonist cells and the pathogen, Possible involvement of the yeast cell-wall material in the inhibition of spore germination and mycelial growth will be discussed.

A501

ANTIFUNGAL EFFECT OF CHITOSAN ON TWO FUNGAL PATHOGENS OF STRAWBERRIES IN VIVO AND IN VITRO. Ahmed El Ghaouth, Rathy Ponnampalam, Joseph Arul and Marcel Boulet. Dept. of Food Science and Technology, Laval University, Ste-Foy, Quebec, G1K 7P4, Canada.

The effect of chitosan coating on strawberries inoculated with Botrytis cinerea or Rhizopus stolonifer stored at 13°C under high humidity - regular stmosphere was investigated. Chitosan coated berries were significantly less decayed after 20 days of storage than the uncoated berries. Chitosan (pH 5.6) at concentrations of 3000 µg/ml and 6000 µg/ml induced leakage of proteins and other U.V. absorbing materials in suspension culture of B. cinerea and R. stolonifer, with a greater effect at the higher concentration. Furthermore, the excessive leakage caused by chitosan at 6000 µg/ml suppressed the ability of fungal tissue for regrowth during 48 hours. It appears that one possible mechanism by which chitosan inhibits fungal infection in strawberries is due to its ability to induce intracellular leakage in fungi.

A502

THE EFFECT OF CHITOSAN ON GROWTH AND MORPHOLOGY OF RHIZOPUS STOLONIFER. Ahmed El Ghaouth, Joseph Arul and Rathy Ponnampalam. Dept. of Food Science and Technology, Laval University, Ste-Foy, Quebec, G1K 7P4, Canada.

The effect of chitosan at pH of 5.6 and 7.2, chemically modified chitosan (O, N, carboxymethylated) and glucosamine on spore germination, germ tube growth, mycelial growth and morphology was studied. Chitosan (pH 5.6) at concentration of 3000 µg/ml was significantly more effective in inhibiting spore germination, germ tube length and radial growth than either chemically modified chitosan, or chitosan (pH 7.2) or glucosamine. Only chitosan (pH 5.6) at 3000 µg/ml induced significant morphological changes such as increased hyphal branching and slower expanding colonies. This work indicates that the antifungal effect of chitosan is attributable to its polycationic nature and polymeric size.

A503

EVALUATION OF OZONE AS A DISINFECTANT IN POSTHARVEST DUMP TANK TREATMENTS FOR TOMATO. J. M. Ogawa, A. J. Feliciano, and B. T. Manji. Plant Path. Dept., Univ. of California, Davis, CA 95616.

Spores of *Mucor piriformis, Botrytis cinerea,* and *Phytophthora parasitica* were exposed to 1.5 - 3.8 µg/ml ozone or 400 µg/ml chlorine solution. Ozone in aqueous solution was prepared by passing pure oxygen through an ozone generator and bubbling gaseous ozone through a column of water. Concentration of ozone was determined with an ozone test kit. Spores of these organisms in a water suspension were inactivated in 20 min at 1.5 µg/ml and in 2 min at 3.8 µg/ml ozone. Spores of *B. cinerea* on the surface of non-injured tomato fruit were inactivated when exposed to 3.8 µg/ml ozone solution for 10 min. The addition of 0.5 g of a loamy soil/L of solution did not affect the ability of ozone to inactivate spores. Spores placed in surface injuries of tomato fruit, however, were not inactivated. In all trials, except injured fruit treatments, 2 min exposures to a chlorine solution inactivated spores of these fungi. Mycelial fragments of *P. parasitica* were more sensitive than *Rhizopus stolonifer* and *M. piriformis* to ozone and chlorine treatments.

A504

DETECTION OF ERGOVALINE IN FESCUE SEED BY ELISA USING A MONOCLONAL ANTIBODY. R. A. Shelby, V. C. Kelley, and G. E. Rottinghaus, Departments of Plant Pathology and Botany and Microbiology, Auburn University, AL 36849 and College of Veterinary Medicine, U. of Missouri, Columbia MO 65211.

Ergovaline (EV) is one of the ergot-peptide alkaloids of fescue infected with the endophytic fungus, Acremonium coenophialum, associated with toxicisis in grazing livestock. A monoclonal antibody to EV was used in a competitive indirect (CI) ELISA of 41 fescue seed lots. Percent fungal infection determined by microscopy varied from 0% to 99%. Accuracy of the CI ELISA in determining EV content was evaluated by HPLC. EV levels determined by both methods were positively correlated with percent infection and ranged from 0-10 µg/g. CI ELISA agreed in all cases with HPLC in terms of the presence or absence of EV, indicating no false negatives or positives, but tended to underestimate EV by about one half in most samples. The CI ELISA provides a rapid screening method for presence and relative concentration of EV, to be used in conjunction with HPLC when more accurate quantitative data are needed.

A505

EFFECT OF BARLEY YELLOW DWARF VIRUS INFECTION ON YIELD AND MALT QUALITY OF BARLEY. B. J. Steffenson[1], M. C. Edwards[2], and P. B. Schwarz[3]. Departments of Plant Pathology[1] and Cereal Science & Food Technology[3], North Dakota State University, and USDA-ARS Cereal Crops Research[2], Fargo, ND 58105.

The adverse effects of barley yellow dwarf virus (BYDV) on yield in barley are well documented; however, little is known about the effect of BYDV infection on malt quality. This study was undertaken to determine the effects of BYDV infection on yield and malt quality of the Midwestern barley cultivars Azure, Morex, and Robust. These cultivars were infected in the field by infesting plants with viruliferous aphids that were reared in the greenhouse. The incidence of BYDV was uniform and at least 99% in aphid infested plots. Compared to control plots, BYDV infection resulted in significant reductions in yield, 1000 kernel weight, and kernel plumpness. Although no significant change was detected in alpha amylase activity, malt extract was decreased and total protein and diastatic power were increased significantly as a result of BYDV infection. These changes in malt quality may be due to a larger percentage of thins in the seed obtained from infected plants; however, preliminary data indicate that other factor(s) may be involved. Thus, BYDV infection of barley results in a reduction in malt quality as well as in yield.

A506

INCIDENCE OF SOILBORNE WHEAT MOSAIC VIRUS AND ITS VECTOR, POLYMYXA GRAMINIS IN FIELD-GROWN SOFT RED WINTER WHEAT. P. T. Himmel, A. D. Hewings, and D. A. Glawe. USDA/ARS and Department of Plant Pathology, University of Illinois, Urbana, IL 61801

Incidence of soilborne wheat mosaic virus (SBWMV) and Polymyxa graminis in four resistant (Hart, Purdue, IL 87-7394, and IL 85-2655) and four susceptible (Cardinal, Rosette, Michigan Amber, and Maryland 75-266-46) soft red winter wheat cultivars was investigated during 1989-1990 in a field trial replicated six times with five subsamples collected per replicate. SBWMV antigen in roots and shoots was detected by enzyme-linked immunosorbant assay (ELISA). P. graminis root infections were noted by light microscopy. From November 1989 until the appearance of symptoms in January 1990, the incidence of SBWMV in shoots of resistant and susceptible cultivars did not differ significantly ($P \leq 0.05$). SBWMV antigen detection in resistant roots was significantly ($P \leq 0.01$) lower in roots of resistant cultivars than in susceptible cultivars. The presence of SBWMV in all plant shoots and the greater frequency of detectible infections in susceptible roots suggest that the mechanism of resistance may be tied to the inhibition of replication or particle assembly resulting in reduced accumulation of virus in resistant roots. Implications of this finding, and the possible relationship to infection of wheat hosts by the fungal vector, will be discussed.

A507

ETIOLOGY OF SOYBEAN SEVERE STUNT DISEASE AND SOME PROPERTIES OF THE CAUSAL VIRUS. T.A.Evans, T. Weldekidan, R.B. Carroll, and R.P. Mulrooney. Dept. of Plant Science, University of Delaware, Newark, DE 19717-1303.

Soybean severe stunt disease (SSSD) is a new virus disease affecting Delaware soybeans. Symptoms occur on first true leaves and infected plants have shortened internodes resulting in severe stunting, thickened dark green leaves, superficial stem lesions and reduced number of flowers and pods. SSSD typically occurs in circular patches in the same fields from year to year and is soil transmitted. There is an association between the occurrence of SSSD and Xiphinema americanum in the field. The causal agent of the disease has been identified as a 28-30 nm isometric virus that is mechanically transmissible to a moderately wide host range. Serological tests against 20 different isometric viruses including most nepoviruses have proven negative. A 3-year survey of soybeans in Delaware indicated SSSD was present in 25 of 40 fields tested and all but one of those fields were located within a 30 square mile area near Millsboro, DE. Initial gel electrophoresis results indicate that the causal agent may not be a nepovirus.

A508

IDENTIFICATION OF CUCURBIT VIRUSES IN COSTA RICA, EL SALVADOR, GUATEMALA AND HONDURAS. J. E. Polston, T. M. Perring, and C. Rivera. University of California, Riverside, CA and University of Costa Rica, San Jose, Costa Rica.

Cucurbits grown in Central America suffer heavy losses due to virus diseases. An investigation into the identity and epidemiology of the major viruses of melon and watermelon was begun in 1988 in El Salvador and Costa Rica, and in 1989 in Guatemala and Honduras. Information on viruses found in melon and watermelon fields will be used in combination with data on insect vector identities, populations and movement to develop a set of management practices designed to reduce virus incidence. Samples for virus assays were collected weekly from approximately 20 locations in each country. Antisera to nine viruses and a potyvirus-specific monoclonal antiserum are being used in indirect ELISAs to screen for viruses. A nucleic acid spot hybridization assay is being used to detect whitefly-transmitted geminiviruses. Results to date indicate that papaya ringspot virus is the most common virus in melons.

A509

ONSET OF MAIZE DWARF MOSAIC IN NORTHERN OHIO. R. Louie and J. K. Knoke, USDA/ARS and The Ohio State Univ., Wooster, OH 44691.

Trap plant (TP) plots with and without diseased source plants (SP), successive plantings, and grass weeds in tile plots were used to monitor maize dwarf mosaic disease (MDM) onset in northern Ohio. TP plots normally detected MDMV in late-August and early-September. TP plots with SP detected MDMV beginning in late-June when SP were placed in the plots. The average incidence of MDM in TP increased from 44 to 52% as the number of SP placed at 0.6 m distance from the TP increased from 25 to 100 plants. At a constant level of 100 SP, the average incidence of MDM decreased from 52 to 33% as the distance between SP and TP increased from 0.6 to 4.9 m. Successive plantings detected MDM onset 31 and 12 days earlier in 1986 and 1987, respectively, than did TP plots. MDMV was not recovered from either the 832 weed samples collected from the field or the six grass weeds grown in tile plots. Rhopalosiphum maidis (Fitch) was significantly related to MDM onset. Aphid migration, seed transmission, and infected weed host hypotheses were evaluated as initial sources of MDMV, but a weed host hypothesis best explained MDM onsets in northern Ohio.

A511

ASSOCIATION OF TOBACCO STREAK ILARVIRUS (TSV) SEED TRANSMISSION AND ANTHER TISSUE INFECTION IN BEANS. M. H. Walter, W. J. Kaiser, R. E. Klein and S. D. Wyatt. Washington State University, Pullman, 99164.

TSV seed transmission was investigated by antigenicity and infectivity assays of flower parts from beans systemically infected with either TSV isolate Mel 40 (Kaiser, Wyatt and Pesho. Phytopathology 72:1508-1512) or TSV isolate Mel F and through reciprocal pollinations. ELISA results indicated that antigen levels of the two virus isolates were similar in flower petals and in ovaries of beans infected with either virus. However, antigen levels in stamen tissues were much lower for TSV F than for TSV Mel 40. The amount of infectious virus (as measured by infectivity assays of flower parts on local lesion host *Chenopodium quinoa*) was also less in stamens of TSV F-infected plants than in stamens of TSV Mel 40-infected plants. Healthy Black Turtle Soup (BTS) bean (*Phaseolus vulgaris* L.) plants were pollinated using anthers from plants systemically infected with either Mel 40 or Mel F. Infected plants were also pollinated using anthers from healthy plants. When Mel 40-infected anthers were used to pollinate healthy plants, 26.38% of the resulting progeny seedlings were infected. Healthy anther X infected ovary pollinations produced 3.36% seedling infection. Mel F isolate was seed transmitted in BTS at less than 0.5%. Seed transmission of TSV in beans may depend on early movement into and replication in pollen-associated tissues.

A512

LEAFHOPPER PROBING PATTERNS ASSOCIATED WITH MAIZE CHLOROTIC DWARF VIRUS (MCDV) TRANSMISSION. A. C. Wayadande & L. R. Nault, Dept. of Entomology, Ohio State University, OARDC, Wooster, OH 44691.

Female Graminella nigrifrons leafhoppers were electronically

monitored on corn during MCDV transmission tests. Control leafhoppers were exposed to and monitored on healthy corn. The controls produced five probing patterns which represent five behaviors; salivation, non-phloem ingestion, phloem penetration (x-waves), phloem ingestion, and non-ingestion. Over half of the leafhoppers on healthy corn initiated phloem penetration and ingestion within 45 min. of the access period. On MCDV-infected plants, more leafhoppers initiated phloem penetration and ingestion than leafhoppers on healthy corn. Fewer MCDV-exposed leafhoppers monitored on test plants initiated phloem ingestion and a significant proportion ingested from xylem. MCDV was transmitted only during phloem penetration and ingestion. These results suggest that specific probing activities associated with phloem ingestion are required for MCDV transmission and that feeding on MCDV-infected corn alters subsequent probing behavior.

A513

VIRION MORPHOLOGY DIFFERENCES BETWEEN TWO TOMATO SPOTTED WILT VIRUS ISOLATES. L. A. Urban, Pi-Yu Huang and J. W. Moyer, Department of Plant Pathology, Box 7616, Raleigh, NC 27695.

Infection of Nicotiana benthamiana by two isolates of tomato spotted wilt virus (TSWV), the common type (TSWV-D), and the impatiens type (TSWV-I), was investigated. Transmission electron microscopy of TSWV-D infected samples revealed electron dense areas, single, spherical enveloped virus particles, and membrane bound enveloped particles. TSWV-I samples also contained morphologically similar electron dense areas, but rarely exhibited single enveloped particles and had no membrane bound particles. TSWV-I samples uniquely contained paracrystalline arrays. All structures of both isolates appeared to be associated with endoplasmic reticulum. Immunogold labeling with whole virus antiserum of the electron dense areas of TSWV-I infected plants have shown these areas to be associated with viral protein. These observations suggest that TSWV-I is defective in virus particle assembly. However, this defect does not affect the disease severity.

A514

PURIFICATION AND PROPERTIES OF CLOSTEROVIRUS-LIKE PARTICLES ISOLATED FROM A CORKY BARK DISEASED GRAPEVINE

S. Namba, D. Boscia, O. Azzam, M. Maixner, J. S. Hu, D. Golino[1] and D. Gonsalves; Plant Pathology Dept., Cornell Univ., NYSAES, Geneva, NY 14456., [1] USDA-ARS, Dept. of Plant Pathology, Univ. of California, Davis, California, 95616.

Closterovirus-like particles were purified from petioles of *Vitis vinifera* cv. Semillon affected with grapevine corky bark disease. Electron microscopy of the purified preparation revealed the presence of flexuous rod-shaped virus-like particles, which were about 13 x 1,400-2,000nm with a helical pitch of 3.4 nm. The molecular weight of the viral coat protein was *ca.* 24 $x10^3$ in Western blotting analysis using specific antiserum. A large dsRNA molecule (*ca.* 10.4 x 10^6) and lower molecular weight species were isolated from bark phloem of corky bark affected Semillon. In ELISA and ISEM tests, antiserum produced to the virus did not react to closterovirus-like particles associated with grapevine leafroll disease (Types II, III, IV) nor grapevine virus A (GVA). Reciprocal tests confirmed these results.

A515

GEOGRAPHICAL ISOLATES OF MAIZE STRIPE VIRUS DIFFERING IN EFFICIENCY OF TRANSMISSION BY, AND TITER IN, THE PLANTHOPPER *PEREGRINUS MAIDIS*. E. D. Ammar[1], R. E. Gingery[2], and L. V. Madden[3]. [1]Dept. of Economic Entomology, Faculty of Agric., Cairo Univ., Egypt, [2]USDA-ARS and Dept. of Plant Pathology, and [3]Dept. of Plant Pathology, The Ohio State Univ.-Ohio Agricultural Res. and Devel. Center, Wooster, OH 44691.

Isolates of maize stripe virus (MStV) from Florida (US), Costa Rica (CR), and Africa (AF), were transmitted to plants by *Peregrinus maidis* (from Hawaii) with respective frequencies of 0, 18, and 60% after a 1-day acquisition-access period (AAP), and 18, 71, and 93% after a 7-day AAP. The isolates were transmitted transovarially to progeny with respective frequencies of 21, 32, and 47%. ELISA of infective planthoppers that had acquired virus orally 4 wk earlier indicated a significantly lower titer of MStV-US compared to -CR or -AF. These results suggest that, compared to the US isolate, the AF and CR isolates reach higher levels in and are transmitted at higher frequencies by *P. maidis* from Hawaii.

A516

SELECTION OF RMV-LIKE ISOLATES OF BARLEY YELLOW DWARF VIRUS EFFICIENTLY TRANSMITTED BY *SCHIZAPHIS GRAMINUM*. D. Hazelwood, S.M. Gray, USDA/ARS, Cornell University, Ithaca, NY, 14853, and T. W. Carroll, Montana State University, Bozeman, MT 59717.

Five isolates of BYDV from Montana (MT) were identified as RMV-like based on serological and aphid transmission assays. Initially they were efficiently transmitted by Rhopalosiphum maidis, and inefficiently by Schizaphis graminum. In 4 serial aphid transfer experiments using both R. maidis and S. graminum in all possible combinations, the probability of transmission by R. maidis for all MT isolates remained high (P=0.9-1.0), regardless of which aphid species inoculated the source plant. Successive transmission by S. graminum resulted in an increase in the probability of transmission (from P = 0.25 to 0.65-1.0) for 3 isolates, while it remained stable (P=0.3) for 2 isolates. In serological assays using antisera to the 5 NY BYDV isolates, the MT isolates reacted only with anti-RMV antiserum. Our findings are in contrast to previous studies on the NY BYDV isolates in which the aphid transmission phenotypes remained stable.

A517

CORN LETHAL NECROSIS IN HAWAII. S. G. Jensen, United States Department of Agriculture, Agricultural Research Service, Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722, J. J. Ooka, University of Hawaii, B. E. Lockhart, University of Minnesota, S. A. Lommel, North Carolina State University, L. C. Lane, D. S. Wysong, and B. Doupnik, Jr., University of Nebraska, Lincoln, NE 68583-0722.

Corn lethal necrosis (CLN), a serious disease of maize, Zea mays, caused by the synergistic action between maize chlorotic mottle virus (MCMV) and a potyvirus, has been positively identified from several dent and sweetcorn fields along the west coast of the island of Kauai, Hawaii. The presence of MCMV was confirmed by symptomatology on maize, by polyacrylamide gel electrophoresis of the capsid protein, and by serology. Serological reactions showed similarities to Nebraska and Kansas isolates. The potyvirus component of the Hawaiian CLN infects both maize and Sorghum bicolor and gives symptoms similar to strains of sugarcane mosaic virus. This finding is important to the seed industry of Hawaii.

A518

1989 CORN VIRUS SURVEY IN SOUTH CENTRAL NEBRASKA AND CORN LETHAL NECROSIS UPDATE. B. Doupnik, Jr. University of Nebraska, Clay Center, 68933, and L. C. Lane and S. G. Jensen, University of Nebraska and USDA-ARS Lincoln, 68583.

Following the severe outbreak of corn lethal necrosis (CLN) in 1988 an intensive corn virus survey was conducted in July and August, 1989 in SC Nebraska. Of the 361 fields surveyed, symptomatic plants were observed and leaf samples collected from 92 fields. These samples were assayed for maize chlorotic mottle virus (MCMV), maize dwarf mosaic virus (MDMV), and wheat streak mosaic virus (WSMV) using gel electrophoresis and host reaction. Of the 92 samples, 38 were positive for MCMV, 12 for WSMV, 5 for MDMV-B and 2 for MDMV-A. One sample was positive for both MCMV and MDMV-B (the synergistic combination that is most commonly associated with CLN) and 34 had no viruses detected. MCMV was widespread in 1989; but, the incidence of MDMV was low and of CLN was extremely low. This is attributed, in part, to the low incidence of greenbugs (the primary vector of MDMV) throughout the 1989 growing season. CLN county records now stand at 9 in SC Nebraska and 7 in NC Kansas.

A519

DEVELOPMENT OF A TRANSFORMATION SYSTEM FOR PEANUT. T. E. Clemente[1], A. K. Weissinger[2], J. W. Moyer[1], and M. K. Beute[1], Departments of Plant Pathology[1] and Crop Science[2] N. C. State University, Box 7616, Raleigh, NC 27695-7616.

Transformation of peanut (Arachis hypogaea) via high velocity microprojectiles to deliver transforming DNA into intact tissues is being investigated. Once a transformation system has been developed utilizing marker genes, it will be employed to introduce gene(s) for potential disease control into the cultivated peanut. Explants from two cultivars, 'Pronto' and 'NC 7', have been tested with two different plasmids, pRT 99 gus, and pBI 221, both of which carry the β-glucuronidase (β-GUS) marker sequence driven by the 35S promoter. Transient expression has been detected by the β-GUS histochemical assay in mature embryos, mature embryonic leaves, callus cultures derived from stem and leaf explants, and mature leaf tissue. Embryonic leaves had the highest degree of transient expression, ranging from 2-12 expressing cells per leaflet.

A520

INDUCTION OF PHENYLPROPANOID METABOLISM DURING A HYPERSENSITIVE RESPONSE IN *ARABIDOPSIS THALIANA*. Keith R. Davis and Farida Shaheen, Department of Botany and the Biotechnology Center, Ohio State University, Columbus, OH 43210

Recent studies by several groups have established the infection of *Arabidopsis thaliana* with phytopathogenic pseudomonads as a model system for studying plant disease resistance. The goal of these studies is to use a combination of biochemical, molecular, and genetic approaches to analyze the complex responses associated with a resistance reaction. Our initial studies have focused on the induction of defense-related genes in cell cultures treated with elicitors, or in leaves infiltrated with virulent or avirulent *Pseudomonas syringae* pathovars. These studies demonstrated that genes involved in phenylpropanoid metabolism, including phenylalanine ammonia-lyase (PAL) and 4-coumarate:CoA ligase (4CL), are induced in elicitor-treated cell cultures and in leaves expressing a hypersensitive response. We have isolated genomic clones of *Arabidopsis* PAL and 4CL genes and are currently analyzing their structure and expression. The results of these studies will be discussed in the context of utilizing genetic approaches for identifying factors involved in activating plant defense responses.

A523

ABSCISIC ACID AS A DETERMINANT OF HOST SUSCEPTIBILITY IN SALINITY PREDISPOSITION TO PHYTOPHTHORA ROOT ROT IN CHRYSANTHEMUM AND TOMATO. R. M. Bostock, J. D. MacDonald, J. M. Duniway, and J. Stites, Department of Plant Pathology, University of California, Davis 95616.

Root stresses resulting from low oxygen, drought, or salinity can lead to increased occurrence or severity of Phytophthora infection. These stresses induce high concentrations of abscisic acid (ABA) in plants. Chrysanthemum roots in hydroponic culture inoculated with Phytophthora cryptogea and exposed to 38 uM ABA for 24 hr prior to transfer to solution without ABA developed disease symptoms as severe as roots exposed to 0.1-0.2 M NaCl. Noninoculated roots exposed to the NaCl and ABA treatments were unaffected. The coupling of ABA and salinity predisposition is being examined using an ABA-deficient mutant (flacca) of tomato, which accumulates about 10% of the root ABA levels present in the wild-type cultivar during salinity stress. Exogenous ABA predisposes roots of both cultivars to severe disease by P. parasitica. Approaches for quantifying disease in tomato roots will be presented.

A524

PROTEINS FROM MAIZE LEAF LEACHATES REDUCE THE SENSITIVITY OF TEXAS MALE STERILE CYTOPLASM MAIZE TO THE HOST SELECTIVE TOXIN FROM BIPOLARIS MAYDIS RACE T. M. O. Garraway, Dept. of Plant Path., OARDC and The Ohio State University, Columbus, OH 43210.

The effect of proteins in maize leaf leachates on the sensitivity of maize to BMT-toxin was determined by infiltrating leaves of Normal (N) and Texas male sterile (T) cytoplasm isolines (cv. W64A) with various dilutions of these proteins mixed with various dilutions of BMT-toxin. Infiltrated leaves were cut into 3 cm pieces, immersed in DDW then the rate of electrolyte leakage was measured. The T but not the N cytoplasm isoline showed increased electrolyte leakage in response to BMT-toxin. This was significantly reduced when T cytoplasm leaves were infiltrated with toxin solutions plus 2 µg/ml or more of proteins from N or T maize leaf leachates. Bovine serum albumin (BSA), a 66 Kd protein, produced a similar response. Binding of maize proteins to toxin or to cell components in competition with toxin may be involved in the reduced activity seen when these polypeptides are mixed with toxin prior to its infiltration into T cytoplasm leaves.

A525

INFLUENCES OF WATER CONGESTION AND XANTHOMONAS CAMPESTRIS PV. PRUNI ON PROTEINS OF PEACH LEAVES. E.L. Bennett and G.E. Carter, Jr. Department of Plant Pathology and Physiology, Clemson University, Clemson, S.C. 29634-0377.

Bacterial spot of peach caused by Xanthomonas campestris pv. pruni (Xcp) is strongly influenced by water congestion of leaves. The effect of water congestion and bacterial infection on leaf proteins was studied. Water congestion was induced by placing plastic bags over cuttings in a dew chamber for 48 h. The plants were spray-inoculated and the bags placed over them for another 24 h. Bags were removed and the plants were sampled 1, 3, 6, and 9 days after inoculation. Proteins were extracted in phosphate-citrate buffer (pH 2.8) and separated by SDS-PAGE. Water congestion caused a decrease or disappearance of some proteins between 21.5 kD and 42.7 kD and below 16 kD as compared to the nonwater-congested plants, while new proteins between 16 kD and 42.7 kD appeared in the plants infected by Xcp. Changes that accompany water congestion may predispose the plants to infection by Xcp.

A526

DETECTION OF PROTEASE PRODUCED BY *Xanthomonas campestris* pv. *zinniae* IN ZINNIA LEAF TISSUE USING THE SUBSTRATE *B*-CASEIN. X.P. Sun and D.F. Ritchie, Department of Plant Pathology, North Carolina State University, Raleigh, 27695.

Zinnia (*Zinnia elegans*) leaves were infiltrated with suspensions of two wild type strains of *X. c.* pv. *zinniae* (Xcz 1 and Xcz 24) and a protease-impaired mutant generated by acridine orange G treatment of wild type strain Xcz 1. Leaf tissue samples were taken 6-72 hr after infiltration, ground in 0.1 M Tris-HCl buffer (pH 8.0), and centrifuged. Supernatants were lyophilized and pellets resuspended in 0.1 M Tris-HCl buffer (pH 8.0). Protease samples were incubated with *B*-casein (5 mg/ml) at 37 C for 3 min and applied to 16% SDS-PAG. The digestion profile of *B*-casein was interpreted as being indicative of the protease in the leaf tissue. No protease activity was detected 6 hr after infiltration of the bacteria into the leaf tissue. After 20 hr, slight protease activity was detected in samples infiltrated with the wild type strains only. At 48 and 72 hr, both wild type strains and the mutant strain digested *B*-casein, but the mutant strain digested less than did the wild type strains. *B*-casein was not digested by extract from the water-infiltrated control, indicating that the zinnia leaf tissue does not contain proteases detectable by this method. This procedure should be useful in detecting and monitoring bacterially-produced protease activity *in planta*.

A527

RESISTANCE TO RACE 2 OF *XANTHOMONAS CAMPESTRIS* PV. *ORYZAE* CONFERRED BY BACTERIAL BLIGHT RESISTANCE GENE *Xa-10* IN RICE INVOLVES LIGNIFICATION OF HOST TISSUES. P.J. Reimers and J.E. Leach. Department of Plant Pathology, Manhattan, KS 66506. U.S.A.

Race-specific resistance (incompatibility) to bacterial blight (BB) of rice caused by *Xanthomonas campestris* pv. *oryzae (Xco)* is correlated with reduced bacterial numbers and shorter lesion lengths in inoculated leaves. When seedling leaves of cultivars carrying the *Xa-10* gene for BB resistance were infiltrated with suspensions of *Xco* (10^9 cfu/ml), a camouflage-brown color began forming in the inoculation site by 18-24 hours after inoculation (HAI) and reached a maximum by 48 HAI, with no water soaking. Compatibility resulted in uniform water-soaking. No response occured after infiltration with water or UV-killed bacteria. Lignin-like materials accumulated throughout the inoculation site in the incompatible interaction, and reached a maximum level by 48-72 HAI. By 120 HAI, lignin-like components were detected only in the perimeter of the compatible interaction. The response, induced by races 2 and 5 of *Xco* on *Xa-10*-containing rice, resembled a hypersensitive response.

A528

CYTOLOGICAL AND BIOCHEMICAL CHANGES OF CELL WALL AS RELATED TO SYSTEMIC RESISTANCE TO BLUE MOLD (*PERONOSPORA TABACINA*) INDUCED BY TMV IN TOBACCO. X. S. Ye, S. Avdiushko, S.Q. Pan, U. Jarlfors, S. Tuzun & J. Kuc. Dept. of Plant Pathology., Univ. of Kentucky, Lexington, Ky 40546.

Inoculation of lower leaves of tobacco Ky14, carrying the *N* gene, with TMV induced systemic resistance to *P. tabacina* and a concomitant systemic accumulation of cell wall hydroxyproline-rich glycoproteins(HRGP). HRGP increased significantly in uninfected leaves 6 days after induction with TMV, and more so after challenge with *P. tabacina*. During this period, HRGP levels in the controls remained unchanged. Four new salt-soluble proteins were detected in the cell wall preparation of the induced plants. These proteins are neither HRGP nor ß-1,3-glucanase or chitinase. Optical microscopy showed that blue mold development in the induced plants was severely restricted, infected cells were plasmolysed, and 4 days after challenge some host cells and fungal hyphae became necrotic near the center of infection sites. Electron microscopy revealed that 4 days after challenge host cell walls in contact with fungal hyphae became more electron-opaque. Electron opaque materials were deposited against host cell walls at haustorial penetration sites, extrahaustorial matrices were much wider, and fungal hyphae often lacked contents in the induced as compared to control plants.

A529

CHITINASE ISOZYME PATTERN AND ITS COORDINATED INDUCTION WITH ß-1,3-GLUCANASE IN TOBACCO PLANTS IMMUNIZED BY PERONOSPORA TABACINA AND TOBACCO MOSAIC VIRUS. S. Q. Pan, X. S. Ye, S. Tuzun and J. Kuć, Department of Plant Pathology, University of Kentucky, Lexington, Ky 40546.

Stem injection of tobacco with sporangiospores of P. tabacina or leaf inoculation with TMV systemically protected plants against diseases caused by both pathogens and systemically increased chitinase and ß-1,3-glucanase activity. Among eight chitinase isozymes detected in tobacco, six did not change even after challenge in protected and control plants. Two were systemically increased in the plants protected by both methods of immunization and accumulated more rapidly in the protected than control plants after challenge with P. tabacina. One of these isozymes was not detected in control challenged leaves prior to 4 days after challenge. When symptoms appeared four days after challenge, the two chitinase isozymes increased in the control plants. The increase of the two chitinase isozymes was coordinated with an increase of a ß-1,3-glucanase and both enzymes were associated with protection.

A530

IDENTIFICATION AND CHARACTERIZATION OF A NEMATODE-SPECIFIC CLASS OF ACETYLCHOLINESTERASE FROM *Meloidogyne arenaria* AND *M. incognita*. Chang, S., and Opperman, C.H. Department of Plant Pathology, North Carolina State University, Raleigh, N.C. 27695-7695-7616.

The site of action of carbamate and organophosphate nematicides in plant-parasitic nematodes is the enzyme acetylcholinesterase. These pesticides block the hydrolytic action of the enzyme at the neuromuscular synapse, resulting in paralysis of the nematode musculature. However, nematodes are able to recover from the effects of carbamate and organophosphate nematicides rapidly, resulting in inconsistent field efficacy of applied compounds. The root-knot nematodes *Meloidogyne arenaria* and *M. incognita* contain a novel class of acetylcholinesterase known to occur only in nematodes. This enzyme has an unusually high affinity for acetylcholine, but is very insensitive to carbamate and organophosphate nematicides. The enzyme purified from *M. arenaria* and *M. incognita* appears to be very similar to Class C acetylcholinesterase found in the free-living nematode *Caenorhabditis elegans*. It is possible that this enzyme in plant-parasitic nematodes is responsible for the rapid recovery from exposure to carbamate and organophosphate nematicides.

A531

USE OF SLOW RELEASE FERTILIZERS FOR CONTROL OF SCLEROTIUM ROLFSII. G. H. Canullo and R. Rodriguez-Kabana. Department of Plant Pathology, Alabama Agricultural Experiment Station, Auburn University, Alabama 36849-5409.

Guanidine thiocyanate, guanylurea sulfate, and thiourea were used in a microplot experiment at 0, 0.25, and 0.5 g/kg soil for control of Sclerotium rolfsii on eggplant (Solanum melongena). Guanylurea sulfate was the most effective compound in terms of reduction of sclerotial production and improvement of plant survival. Guanylurea sulfate was tested in combination with guanidine thiocyanate or thiourea under greenhouse conditions. The combination guanylurea sulfate + guanidine thiocyanate was the most successful for controlling S. rolfsii. The combination treatment of 0.15 g guanylurea sulfate + 0.05 g guanidine thiocyanate/kg soil was the lowest effective rate.

A532

ENHANCED SOYBEAN PLANT GROWTH AND NODULATION BY BRADYRHIZOBIUM IN THE PRESENCE OF STRAINS OF BACILLUS MEGATERIUM. Z. L. Liu and J. B. Sinclair, Dept. of Plant Pathology, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801-4709.

Enhanced soybean plant growth and nodulation by Bradyrhizobium was obtained in nutrient culture in a controlled environmental chamber in the presence of Bacillus megaterium ATCC-55000 and M2144. Seedlings from seeds coated with Bradyrhizobium plus either 55000 or M2144 had significantly higher plant dry weight, nodule dry weight, nodule number, and nitrogenase activity than the control. None of the growth parameters was significantly increased above the control when the two strains were applied separately as a solution treatment at V1 growth stage. Both strains used as seed treatments with Bradyrhizobium resulted in denser and more widely distributed nodules compared to the control. The mechanism involved in this enhancement appeared not to be the same as that in antagonism to Rhizoctonia solani, since M2144 was an antagonist-deficient mutant. The nodulation enhancement was recorded in the field at V8 to R1 growth stages but yield effects varied.

A533

ROLE OF 2,4-DIACETYLPHLOROGLUCINOL IN DISEASE SUPPRESSION BY A STRAIN OF *PSEUDOMONAS FLUORESCENS*. C. Keel[1], C. Voisard[2], D. Haas[2], and G. Défago[1], Departments of [1]Plant Sciences/Phytomedicine and of [2]Microbiology, Swiss Federal Institute of Technology, 8092 Zürich, Switzerland.

Pseudomonas fluorescens strain CHA0, which is an effective biocontrol agent of soilborne plant pathogens, produces several toxic metabolites, notably cyanide, acetylphloroglucinols and pyoluteorin. By genetic manipulation of strain CHA0, cyanide was shown to be an important factor in the suppression of black root rot of tobacco caused by *Thielaviopsis basicola*. Strain CHA625, which was obtained after Tn*5* mutagenesis, did not produce 2,4-diacetylphloroglucinol and suppressed black root rot of tobacco and *Gaeumannomyces graminis* var. *tritici*-induced take-all of wheat to a distinctly smaller extent than did wild-type CHA0 under gnotobiotic conditions. A cosmid, pME3101, obtained from a genomic library of strain CHA0 restored the ability of strain CHA625 to produce this metabolite and partially restored its suppressive capacity. 2,4-Diacetylphloroglucinol was shown to be produced by strains CHA0 and CHA625/pME3101 but not by strain CHA625 in the rhizosphere of wheat, grown under gnotobiotic conditions. These results suggest that the production of 2,4-diacetylphloroglucinol by strain CHA0 plays an important role in the suppression of soilborne plant pathogens.

A534

MECHANISMS OF RESISTANCE TO ROOT-LESION NEMATODE (PRATYLENCHUS PENETRANS) IN ALFALFA. J.A. Thies, D.H. Basigalup, D.K. Barnes, and R.D. Wilcoxson, USDA-ARS and Depts. of Plant Pathology and Agronomy & Plant Genetics, Univ. of Minn., St. Paul, MN 55108.

Resistance of alfalfa (Medicago sativa) to Pratylenchus penetrans has been reported, but mechanisms of resistance have not been determined. Rooted ramets of 25 alfalfa clones inoculated with P. penetrans were grown for 6 weeks in free-choice and no-choice tests at 25C with a 16 hr photoperiod. An equal number of noninoculated ramets served as controls. The clones varied for numbers of nematodes/plant, nematodes and eggs/g fresh plant weight, and for percent fresh plant weight (plant weight of inoculated clone/plant weight of noninoculated clone). Resistance mechanisms identified were antibiosis (characterized by low numbers of nematodes in roots) and tolerance (characterized by large plant weights and moderate to high numbers of nematodes in roots). Nematodes + eggs/g fresh plant weight and percent fresh plant weight of control were the most important criteria for selecting clones with highest degrees of antibiosis and tolerance, respectively.

A535

HEAT INACTIVATION OF GAEUMANNOMYCES GRAMINIS VAR. TRITICI (GGT). W. W. Bockus and B. L. Norman, Department of Plant Pathology, Kansas State University, Manhattan 66506-5502.

Inoculum of GGT was exposed to 35, 40, or 45 C for various time periods to determine the inactivation point. Exposure occurred in moist (-0.1 MPa), autoclaved and nonautoclaved field soil in petri dishes. Natural inoculum (infected wheat crowns from the field) and artificial inoculum (colonized oat kernels) were compared. After exposure, inoculum was used in a bioassay to quantify the ability of GGT to cause root rot and losses in fresh weight on wheat seedlings. Inactivation at 35 C required 10-15 exposures of 6 hr/day (depending upon the experiment); at 40 C, inoculum was inactivated after 3-5 exposures of 6 hr/day; and at 45 C, it was inactivated after a single exposure of 2.5-5 hr. No significant differences were detected between the response in autoclaved vs. nonsterile soil; however, natural inoculum was slightly more resistant to inactivation than artificial inoculum. Results of these experiments suggest that inoculum of GGT is sensitive to heat inactivation and may explain why it does not survive in bare soil for 8 wk during the summer in Kansas.

A536

CORRESPONDENCE OF RHIZOSPHERE COMPETENCE ON MAIZE TO CARBON UTILIZATION WITH SIX Fusarium SPECIES. Cynthia M. Ocamb and Thor Kommedahl, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Fusarium moniliforme, F. oxysporum, F. proliferatum, and F. solani extensively colonize the rhizosphere while F. equiseti and F. graminearum are not rhizosphere competent. Enzyme production for carbon utilization may reflect rhizosphere competence. All six species of Fusarium grew on various carbon substrates with Czapek-Dox salts: cellobiose, cellulose, galactose, pectin, xylan, and xylose. Sporulation and mycelial dry weight were evaluated on cellulose, pectin, and dextrose. Fusarium moniliforme generally sporulated more than other species, especially on the dextrose and pectin substrates. Cellulose yielded significantly greater mycelial dry weights than pectin or dextrose. Mean mycelial dry weight produced on cellulose, pectin, and dextrose was greatest by F. graminearum and generally least by F. solani. Rhizosphere competency of these six Fusarium species appears not to be related to utilization of cellulose or pectin.

A537

EFFECT OF NACL ON CARBOHYDRATES AND MALATE PRODUCTION IN ASPARAGUS ROOTS AND ON INFECTION BY FUSARIUM. W. H. Elmer, Dept. of Plant Pathology and Ecology, The Connecticut Agricultural Experiment Station, Box 1106, New Haven, Connecticut 06504.

Asparagus plants, grown in sand culture in the greenhouse, received biweekly applications of 0, 0.1, 0.3, 0.5, or 1.0 g of NaCl dissolved in 100 ml of Hoagland's solution. Half of the pots in each NaCl treatment were inoculated with 100 ml of H_2O containing 10^8 conidia of F. oxysporum and 10^8 conidia of F. moniliforme. The other pots received 100 ml of sterile H_2O and served as noninoculated controls. The percentage of roots with lesions (RL) and root colonization (cfu/cm root) (RC) were measured in inoculated plants after 3 mo. Disease (RL, RC) decreased as NaCl rates increased. Levels of carbohydrates significantly increased and malate significantly decreased in noninoculated roots treated with increasing rates of NaCl. Because these rates of NaCl did not affect the growth of these Fusaria in culture, it is postulated that NaCl suppresses disease by mechanisms associated with malate and carbohydrate metabolism.

A538

EFFECT OF IRRIGATION, FUMIGATION, AND SULFUR ON THE DEVELOPMENT OF STREPTOMYCES SOIL ROT AND YIELD IN SWEETPOTATO. J. B. Ristaino, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Sweetpotato plots infested with Streptomyces ipomoea were either not irrigated or drip irrigated during the season. Subplots were treated with sulfur (90 W 333lb/A) and sub-subplots were fumigated with Telone C-17 (10.5 gal/A) prior to transplanting. Soil pH was reduced from 5.8 to 5.2 in sulfur-treated plots. Fumigation increased yields by 58% and decreased disease on storage roots by 27%. Highest yields and lowest severity of disease on fibrous roots occurred in plots that were fumigated, irrigated and treated with sulfur. Fumigation increased the number of storage roots produced per plant, while fumigation and sulfur reduced the number of diseased storage roots produced per plant. Irrigation alone did not affect disease on storage roots but reduced disease on fibrous roots in combination with fumigation and sulfur. Management of soil rot requires an integration of chemical and cultural practices.

A539

DETETECTION OF *PHYTOPHTHORA* SPECIES IN CRANBERRY FIELD SOILS. M. J. Drilias and S. N. Jeffers, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Phytophthora species have been associated with a decline syndrome of cranberry in Wisconsin. A baiting bioassay was developed to detect *Phytophthora* spp. in cranberry field soils. Succulent cuttings, 5.5-6.5 cm in length, from the actively growing, terminal portions of cranberry stems (cv. Stevens) were used as baits. A 65-ml aliquot of soil, sieved through a 6-mm screen, was placed in a 150-ml, wax-coated paper cup. Three to four baits were placed in each cup so that the basal 4.0-4.5 cm of each stem was buried in soil. Soils then were flooded by adding enough distilled water to provide a 1.0- to 1.5-cm layer of water over the soil surface. Baited soils were placed at constant temperatures for up to 2 wk. Baits were examined every 2-3 days, and those with discolored stems were plated onto a medium selective for *Phytophthora* spp. Wetting soils to and then maintaining them at field capacity for 3 days prior to flooding and baiting significantly increased the number of baits colonized by *Phytophthora* spp. The number of baits colonized at 16 C was comparable to that at 20 C and was significantly greater than that at 24 C. *Phytophthora* spp. were not detected in soils baited at 28 C. Isolates of *Phytophthora* spp. recovered from field soils were morphologically similar to those previously isolated from cranberry plants.

A540

AN ISOLATE OF PHYTOPHTHORA ARECAE FROM FLORIDA PATHOGENIC TO CITRUS. L. W. Timmer, S. E. Zitko, and H. A. Sandler, University of Florida, Citrus Research and Education Center, Lake Alfred 33850.

A Phytophthora sp. recovered from soil in a citrus orchard near Ft. Pierce most closely fit the description of P. arecae (probably synonymous with P. palmivora). It produced papillate, caducous sporangia averaging 50 μm long and 30 μm wide with a pedicel 3 μm long. All isolates were A^1 and mated readily with A^2 types of P. parasitica and P. palmivora. Oogonia, oospores, and chlamydospores averaged 30, 25, and 34 μm in diam., respectively. Optimum temperature for growth was 30 C with little or no growth at 15 or 33 C. The citrus isolate of P. arecae (P.a.-citrus) was as pathogenic as P. parasitica to fibrous roots of sweet orange, sour orange, and Swingle citrumelo, but a palm isolate (P.a.-palm) was not pathogenic to roots. The P.a.-citrus and the P.a.-palm isolates were pathogenic to fruit. The P.a.-citrus, P.a.-palm, and P. parasitica produced stem lesions on Etrog citron cuttings. This is the first report of a P. arecae pathogenic to citrus in the U.S.

A541

REDUCTION OF SEEDLING EMERGENCE DUE TO RHIZOCTONIA ZEAE IN TWO TALL FESCUE VARIETIES. K.D. Gwinn and A.M. Gavin. University of Tennessee, Knoxville, TN 37901-1071.

Reduction of seedling emergence due to Rhizoctonia zeae (NC RZ112J) was examined in two tall fescue varieties which differ in endophyte (Acremonium coenophialum) levels. Endophyte levels were determined to be 85% ('Kentucky-31') and <5% ('Forager'). Seed (10 g/flat) were planted in flats of either Promix or Promix amended with R. zeae (NC RZ112J). Number of seedlings/5 cm core was determined; 3 cores/flat were counted. Five flats/treatment were used and the experiment was repeated three times. Seedlings/core in nonamended Promix was different ($P<.001$) from seedlings/core in Rhizoctonia-amended Promix for both varieties. For Kentucky-31, seedling emergence in amended soil was 70% of emergence in nonamended soil, however for Forager, seedling emergence was reduced to 36% in amended soil. Whether differences are due to endophyte infection or genotype will be investigated.

A542

MOVEMENT OF PHYTOPHTHORA ZOOSPORES THROUGH COLUMNS OF CONTAINER MEDIA. D. M. Benson, Dept. of Plant Pathology, N. C. State Univ., Raleigh 27695.

Zoospores of *P. parasitica* were added to 15-cm-high columns of saturated pine bark (PB), pine bark:sand (PBS, 3:1), or peat:sand:soil (PSS, 1:1:1) media. The void volume (vv) was collected and cultured on a PARP medium for colonies. Void volumes were 70, 40, and 10 ml, for PB, PBS, and PSS, respectively. Additional void volume equivalents were added to each column and collected. For motile zoospores, maximum counts (range 100-320/vv) were found in the 2nd, 2nd or 3rd, and 7th or 8th void volumes for PB, PBS, and PSS, respectively. For encysted zoospores, less than 20 zoospores/vv were recovered from any void volume or medium. Large pores with rapid drainage probably account for differences in rate of movement of motile zoospores in pine bark media compared to PSS. Encysted zoospores apparently were trapped in small soil pores in all media. Phytophthora root rot may not be as severe in pine bark media which allows rapid movement of zoospores through the medium.

A543

RELATIONSHIP BETWEEN DEVELOPMENT OF PHYTOPHTHORA ROOT ROT AND YIELD IN COMMERCIAL FIELDS OF PROCESSING TOMATO. D. Neher and J. M. Duniway. Dept. of Plant Pathology, University of California, Davis, CA 95616.

Six-meter-long rows of tomato variety 6203 were planted adjacent to five other varieties in several fields with a history of Phytophthora root rot. Above-ground symptoms of disease developed at a similar phenological stage in all varieties and the final disease incidence and severity ranged from zero to the maximum possible. The harvestable fruit decreased linearly with increased symptom severity in eight plots of 6203 in one field, and all other varieties and sites fit the same relationship within 95% confidence limits. Phytophthora parasitica was not detected by preplant soil dilution plating at all sites where disease became severe, but was detected by a baiting method. Final disease severity was correlated positively with soil clay content and cation exchange capacity and negatively with soil sand content.

A544

EFFECT OF SOIL MATRIC POTENTIAL ON INFECTION OF PEPPER BY OOSPORES OF PHYTOPHTHORA CAPSICI. M. J. Hord and J. B. Ristaino, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

Pepper seedlings were grown in microwaved soil infested with 50 oospores/g dry soil in tension funnels. Soil was

maintained at 0, -25, -50 or -100 mb for 5 or 10 days prior to a 2 or 24 hr saturation period. Above ground symptoms were recorded and final disease incidence was determined by isolation on a selective medium 10 days after saturation. A saturation period was necessary for infection. Longer incubation prior to saturation resulted in more rapid disease progress and greater disease incidence. Final disease incidence after the 2 hr saturation was 48, 45 and 58% with the 5-day incubation period, and 85, 100 and 95% with the 10-day incubation period at -25, -50 and -100 mb, respectively. With constant saturation, 20% of the plants became infected. Infection of leaf disks during the saturation periods indicated indirect germination of oospores occurred in all treatments.

A545

A FLUORESCENT PSEUDOMONAS SPP. ASSOCIATED WITH A NEW LEAF BLIGHT AND BULB ROT OF VIDALIA ONIONS IN GEORGIA. R. Gitaitis[1], R. Baird[1], R. Beaver[1], D. Gay[1], D. Sumner[1], and D. Smittle[2]. Depts. of Plant Pathology[1] and Horticulture[2], University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793.

A foliar blight of onion (Allium cepa) resulting in a wet rot at the base of the leaves and of one or more inner scales of the bulb was observed for the first time in Georgia. A fluorescent-pigmented bacterium similar to Pseudomonas viridiflava was consistently recovered from all samples. The bacterium was negative for oxidase and arginine dihydolase, and variable for HR in tobacco. It rotted potato and carrot slices, and degraded sodium polypectate gel at pH 8.5 and in CVP but not at pH 5.0. However it slowly utilized sucrose and weakly produced levan. Inoculation of greenhouse-grown onion plants with the bacterium resulted in the reproduction of the same symptoms observed in the field and in the recovery of a fluorescent bacterium with the same traits. A pectolytic Xanthomonas spp. also was recovered from many but not all field specimens.

A546

SURVIVAL OF *COLLETOTRICHUM COCCODES* IN NEW YORK. Helene R. Dillard, Department of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456.

Sclerotia of *C. coccodes* and colonized tomato skin (fruit) tissue were enclosed in pouches made of Nitex fabric (155 *um* mesh) and placed at 0, 10 and 20 cm depths in soil on 11/15/88. The pouches were located at 49 sites in a 23 m strip in a 0.4 ha field and flagged. At regular intervals, a pouch was removed from each depth and sclerotia and tomato skin pieces were assessed for viability of *C. coccodes*. After 469 days, *C. coccodes* was isolated from 100, 96, and 92% of the skin tissues and 100, 94, and 96% of the sclerotia were viable at the 0, 10, and 20 cm depths, respectively. In a separate experiment, sclerotia of *C. coccodes* and colonized tomato stem and skin tissue were placed in natural field soil (16% moisture by weight) and incubated at 7, 16, 25, and 31 C. After 477 days incubation, survival of *C. coccodes* in skin and stem tissues and as sclerotia was >50% at all temperatures.

A547

WHITE MOLD (SCLEROTINIA SCLEROTIORUM) IN FLORIDA CABBAGE DURING 1989-90. D. P. Weingartner, AREC, Hastings, FL 32145.

Studies of a white mold epidemic on a 140 ha commercial cabbage farm were reinitiated in mid-October, 1989. Ascosporic inoculum was present the entire season. Apothecia were found either in cover crop or cabbage fields from 8 November to April. They were most prevalent in December. White mold developed during mid-November to April in different sets of potted bean plants following 4-7 day exposures to field inoculum. The first sign of ascosporic infection in cabbage was observed 1 December. Disease incidence increased slowly in December followed by a rapid increase after the hard freeze of 23-26 December. The percentage disease in 2 fields increased from 1.08% and 0.04% 21 December to 37.9% and 87.4% 11 and 12 January, respectively. Similar increases in white mold following the freeze occurred throughout Florida. The disease increase in 11 of 13 fields was due to ascospores whereas infection in 2 was mostly from the soil. The mechanism of the increase in white mold is unknown but may result from freeze injury and/or induction of carpogenic and/or mycelial germination of sclerotia.

A548

REACTION OF SPINACH CULTIVARS TO A NEW RACE (RACE 4) OF DOWNY MILDEW IN THE UNITED STATES. **L.P. Brandenberger, J.C. Correll, and T.E. Morelock*. Dept. of Plant Pathology and *Dept. of Horticulture and Forestry, University of Arkansas, Fayetteville, AR. 72701.**

A new race (race 4) of Peronospora farinosa f. sp. spinaciae was identified in California and Texas. The differentials used for race identification included Viroflay (susceptable to races 1,2,3), Nores (resistant to races 1 and 2), Califlay (resistant to races 1 and 3) and Polka and St. Helens (resistant to races 1,2,3). All of the differentials were susceptible to race 4. All five isolates recovered in California were identified as race 4. Of the four isolates recovered from the Winter Garden area of Texas, one isolate was identified as race 4 and three isolates were identified as race 3. Replicated growth chamber inoculation tests on 26 commercial cultivars and five Arkansas breeding lines were carried out using a single isolate of race 4 (from California) and a single isolate of race 3 (from Washington). Of the 26 cultivars tested, 17 have reported resistance to races 1,2 and 3, seven have reported resistance to races 1 and 2 and two have reported resistance to races 1 and 3. Two of the Arkansas breeding lines have reported polygenic resistance to race 3. All cultivars and breeding lines tested to date were susceptible to race 4. Plant introductions of spinach are also being evaluated; those tested thus far have proven to be susceptable.

A549

A PROGRAM OF TESTING NEW AND STANDARD CELERY CULTIVARS FOR RESISTANCE TO YELLOWS DISEASE INCITED BY Fusarium oxysporum f. sp. apii. A.S. Greathead. Farm Advisor Emeritus, Univ. of California Cooperative Extension, Salinas, California 93908

Celery yellows caused by Fusarium oxysporum f. sp. apii. has resulted in serious losses to celery growers in all the major production areas of California. The release of the cultivar UC 1 by the University of California has provided the industry with a highly resistant but horticulturally unsatisfactory line. A program of field testing of commercial and experimental cultivars in the Salinas Valley of California was developed in 1987 and has continued through 1989. Reliable ratings of the resistance levels of a number of lines have been developed. Two new cultivars - Matador and Starlet - have proved to be highly resistant to yellows and to be satisfactory horticulturally. Other lines not yet available for commercial use are showing merit.

A550

PESTS OF CAPER, CAPPARIS SPINOSA -- SOME NEW RECORDS FOR CALIFORNIA. D. G. Kontaxis, Cooperative Extension, University of California, 1700 Oak Park Blvd., Bldg. A-2, Pleasant Hill, CA 94523.

Caper, Capparis spinosa, a member of the Caparales order, grows well in the Mediterranean area, where it is commercially cultivated. Caper is used as food condiment (herb) and vegetable, and also as an ornamental. The U.S. imports about $20 million worth of caper every year from Spain, Morocco, Israel and other countries. Caper is practically unknown in the United States. Caper plants were planted in 1988 and 1989 to study crop adaptability and pest disorders. In California the fungi, Botrytis sp. and Pythium sp., insects, Pieris rapae and Brachyrhinus sulcatus, attacked caper plants. These are new records for this host and pests in California.

A551

INTERACTIONS BETWEEN PURPLE BLOTCH AND ONION THRIPS ON BULB YIELD. Marvin E. Miller, Texas Agricultural Experiment Station, 2415 E. Highway 83, Weslaco, TX 78596 and Jonathan Edelson, Oklahoma State University, P.O. Box 128, Lane, OK 74555.

Interactions between purple blotch (Alternaria porri) and onion thrips (Thrips tabaci) on bulb yield were determined for onion cvs. Texas Grano 1015Y (TG 1015) and Ben Shemen (BS). Purple blotch severity levels were maintained by weekly treatments of either iprodione at 1.12 kg (ai)/ha, anilazine at 1.12 kg (ai)/ha, mancozeb at 2.69 kg (ai)/ha or no fungicide to onion plots. Number of thrips were maintained by either applying cypermethrin to plots at 0.11 kg (ai)/ha when populations reached 0-5, 5-10, 10-25 thrips per plant or no insecticide. TG 1015 and BS yields were significantly ($p=0.01$) affected by purple blotch severity levels but thrips populations affected yields only on BS. Severity of purple blotch was significantly higher at the highest thrips population level. There were no significant interactions between purple blotch and thrips on yield.

A553

DEVELOPMENT OF BLACK ROOT ROT (THIELAVIOPSIS BASICOLA) AS A POST-HARVEST DISEASE ON FRESH MARKET CARROTS AND STRATEGIES FOR DISEASE CONTROL. Zamir K.Punja, Centre for Pest Management, Dept. of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6.

Black root rot, caused by Thielaviopsis basicola (Berk.and Br.)Ferr. (= Chalara elegans Nag Raj and Kendrick) was a severe post-harvest disease on fresh market carrots grown in muck soils in the Fraser Valley of B.C. in 1989. Symptoms appeared at retail outlets as black lesions on the root; the pathogen was most destructive when carrots were stored in polyethylene bags at temperatures above 18 C. Chemical salt dips were compared with the recommended NaOCl treatment for effectiveness in preventing disease. Roots were inoculated with T.basicola 48-72 hr prior to, and immediately after, each treatment and incubated at 19-21 C for 7-10 days. Among five salts, calcium propionate (1% aqueous solution for 60-90 sec) reduced infection to less than 5% of the control (40% of root colonized) if applied within 48 hr post inoculation or immediately preceeding inoculation. Potassium carbonate, sodium bicarbonate, sodium formate, and NaOCl (each at 1% concentration) provided decreasing levels of disease control, respectively.

A555

INHERITANCE OF TOMATO SPOTTED WILT VIRUS (TSWV) RESISTANCE IN TOMATO. J. J. Cho, J. C. Watterson, C. Wyatt, and D. M. Custer, University of Hawaii, Maui Research, P. O. Box 269, Kula, HI 96790, and Petoseed Co, Inc., Rt 4, Box 1255, Woodland, CA 95695.

Inheritance of TSWV resistance have been tested in inbred resistant tomato lines from an *L. peruvianum* X *L. esculentum* cross. TSWV inheritance appears to be controlled by a single dominant gene which is simply inherited. Plants of the F1 generation from crosses between susceptible and resistant parents exhibited local lesions, but the virus failed to develop systemic infections, revealing that resistance was dominant. F2 populations also responded with local lesions, however, only approximately 25 % of the plants became systemically infected, indicating that resistance was monogenic. Plants of the F1 X resistant parent backcross segregated in the ratio of one systemic resistant to one susceptible (1:1) giving further confirmation to inheritance of a single dominant gene for TSWV resistance.

A556

SENSITIVITY OF LACTUCA SATIVA AND CICHORIUM ENDIVIA TO FOSETYL-AL FOLIAR APPLICATIONS. M. L. Sommerfeld and R. N. Raid, A. Duda and Sons, Inc., and U. of Florida, Everglades Research and Education Center, Belle Glade, FL 33430.

Aerial application of fosetyl-Al is currently the most widely used method of controlling lettuce downy mildew in Florida. Five lettuce cultivars, escarole, and endive were tested for sensitivity to simulated aerial fosetyl-Al applications in a replicated field trial. A commercially-available formulation and a pH-buffered formulation were applied to foliage alone and in combination with a copper fungicide and Techmangam, a micronutrient product. Significant phytotoxicity was observed across all leaf types when the nonbuffered formulation was tankmixed with copper fungicide. Nonbuffered fosetyl-Al alone resulted in slight phytotoxicity on all leaf types except escarole. Nonbuffered fosetyl-Al and Techmangam tankmix applications resulted in only slight phytotoxicity on romaine and bib lettuce types. Use of the buffered fosetyl-Al formulation reduced phytotoxicity to near nondetectable levels in all combinations across all leaf types.

A557

FIRST REPORT OF RESISTANCE OF *HELMINTHOSPORIUM SOLANI* TO THIABENDAZOLE IN THE UNITED STATES. C. L. Merida and R. Loria, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Helminthosporium solani was isolated from tubers with silver scurf collected from five locations in New York State. Eight single-spore isolates were grown on V8 agar amended with 1, 3.2, 10, 31.2 or 100 mg thiabendazole (TBZ)/L, and radial growth was measured after 20 and 40 days at 21 C. Two of the eight isolates tested grew at concentrations up to 31.2 mg TBZ/L. The remaining isolates did not grow at concentrations greater than 1 mg TBZ/L. These results were repeatable. Fungicide dose response curves were constructed for each of the resistant isolates. The TBZ concentration that reduced growth of the resistant isolates by 50% (25 mg TBZ/L) was utilized to screen 50 isolates that had been obtained from diseased potato tubers. Twelve isolates from three locations within New York State were resistant to TBZ. Average growth rate of six resistant isolates was 1.2 times larger than that of two sensitive isolates on unamended V8 agar after 45 days incubation at 21 C.

A558

VERMIAL VIRUCIDAL ACTIVITY : IMPLICATIONS FOR MANAGEMENT OF PATHOGENIC BIOLOGICAL WASTES ON LAND. L. S. Amaravadi, M. S. Bisesi, R. F. Bozarth, Indiana State University, IN-47809.

The earthworm Eisenia fetida, known to contain bactericidal enzymes, was tested for virucidal activity using CPMV and TMV as models agents. Earthworms were fed cellulose saturated with a virus suspension and their excreted castings were analyzed by ELISA and local lesion assays. Our results indicated a considerable reduction in infectivity of both viruses. Virucidal activity was also observed when virus suspensions were incubated with the earthworm enzyme extract. The observed reductions in the infectivity of both viruses suggest that E. fetida may possess a virucidal enzyme system and, accordingly, may contribute to the inactivation of pathogenic viruses potentially associated with land application of sewage sludges and livestock manures.

A559

THE EFFECTS OF O_3 AND ACID RAIN ON ECTOMYCORRHIZAL COLONIZATION OF PINUS TAEDA L., Z. Qiu, A.H. Chappelka, G.L. Somers, B.G. Lockaby, R.S. Meldahl, F.C. Thornton and J.S. Kush, School of Forestry, Auburn University, AL 36849, and TVA, Muscle Shoals, AL 35660.

Pinus taeda L. seedlings from 2 families differing in ozone sensitivity were exposed to 4 O_3 conc. (sub-ambient, ambient, ambient x 1.7 and ambient x 2.5) and 3 levels of acid rain (pH = 3.3, 4.3, 5.3) in modified open-top chambers for one growing season. Seedlings were planted in root exclusion tubes to isolate root systems of individuals. At harvest, the trees were excavated and root systems separated. Subsamples were evaluated for presence of non-mycorrhizal and mycorrhizal short roots. Mycorrhizae were separated into groups based on surface morphology. There were no sig. diff. in the presence of non-mycorrhizal short roots and individual mycorrhizae cm^{-1} among treatments. Total numbers of morphotypes increased with increasing O_3, but numbers of mycorrhizal tips cm^{-1} did not vary among treatments.

A560

OCCURRENCE AND EXPRESSION OF SORGHUM YELLOW BANDING VIRUS IN SOUTH TEXAS. G. N. Odvody, R.W. Toler*, and J. Remmers. Texas A&M Expt. Station, Corpus Christi, TX 78410. *Texas A&M University, Department of Plant Pathology and Microbiology, College Station, TX 77843

By 1989 Sorghum Yellow Banding Virus (SYBV) was observed in 1 county nonadjacent to and 7 counties contiguous with the initial site of observation near Floresville in 1984. SYBV occurred naturally only on sorghum x sudan grass

hybrids except for one occurrence on grain sorghum tillers. No SYBV was observed on primary growth and initial or highest incidence was generally observed at field perimeters on basal rather than stem tillers. In an irrigated field, incidence of SYBV increased with each of two ratooned crops (< 1% and 10-30%). The slow recovery single ratoon crop of several dryland fields had a higher incidence of SYBV (10-25%) than the comparable irrigated ratoon crop. SYBV occurred in the third and subsequent ratoon crops of sorghum x sudan in greenhouse tests using soil from an SYBV-affected field but not another field soil. Aphids did not transmit the virus and vectors are yet unknown but results indicate a soilborne mechanism for SYBV.

A562

PATHOGENICITY OF FUNGI ASSOCIATED WITH ALFALFA STEM NEMATODE INFESTATIONS OF COLORADO ALFALFA. Pickett, L. S. and W. M. Brown, Jr. Jefferson County Extension, Golden, CO 80401 and Dept of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523, respectively.

Colorado alfalfa infested with the alfalfa stem nematode, Ditylenchus dipsaci, were also found to be infected with one or more of the following fungi: Fusarium oxysporum, F. equisti, F. solani, and Rhizoctonia solani. Pathogenicity studies with fungi associated with nematode infestations have shown that these fungi are crown rotting organisms capable of producing disease symptoms only when host plants are under drought stress.

A563

CERCOSPORA LEAFSPOT OF SUBTERRANEAN CLOVER IN SOUTH TEXAS. R. G. Pratt, USDA, ARS, P.O. Box 5367, Mississippi State, MS 39762, and W. R. Ocumpaugh, Texas Agr. Exp. Sta., HCR-2, Box 43-C, Beeville, Tx 78102.

Cercospora leafspot symptoms were observed on cultivars and introduced lines of subterranean clover in a reseeding nursery at Beeville, Texas, during 1988 and 1989. Isolates of the pathogen, identified as Cercospora zebrina, were compared with isolates from Mississippi for pathogenicity on subterranean clover, other clover species, and alfalfa. Subterranean clover cultivars Daliak, Dwalganup, Mt. Barker, and Woogenellup were highly susceptible to both Texas and Mississippi isolates whereas Clare and Yarloop were resistant. Few or no symptoms were induced on arrowleaf, berseem, crimson, red and white clovers and alfalfa by Texas and Mississippi isolates. Symptoms on subterranean clover developed more rapidly at 26 and 22 C than at 18 C. These results document the first known occurrence of a host-specific strain of C. zebrina on subterranean clover outside of Mississippi and indicate that isolates from both Texas and Mississippi are similar in pathogenicity.

A564

ALFALFA MOSAIC (AMV), CUCUMBER MOSAIC (CMV), AND BEAN YELLOW MOSAIC (BYMV) VIRUSES INFECTING ANNUAL MEDICS (MEDICAGO SPP.). W. Pathipanawat, R.A.C. Jones, and K. Sivasithamparam, Western Australian Department of Agriculture, South Perth, WA 6151 and The University of Western Australia, Nedlands, WA 6009; AUSTRALIA.

Seeds of annual medics collected from plants separately inoculated with AMV, CMV and BYMV were found to transmit all three viruses. AMV was transmitted at the highest level (e.g. murex medic (M.murex) at 18-65%) compared to CMV (0.3-1.5%) and BYMV (0.9-1.1%). Pollen from AMV and BYMV infected plants was transferred to healthy plants and vice versa. Tests on seedlings from these crosses failed to detect virus transmitted through pollen. Seedlings grown from seeds collected from an AMV infected plant of murex medic cv. Zodiac, but receiving healthy pollen, were infected, indicating that AMV infected the ovary. In screening for AMV resistance, button medic (M.orbicularis) accession DZA 3177.1 resisted virus infection by sap, aphid and graft inoculation.

A565

EFFECT OF CULTIVAR RESISTANCE AND INOCULUM POSITION ON ROOT INFECTION AND SYMPTOMS CAUSED BY PHYTOPHTHORA PARASITICA VAR. NICOTIANAE ON NICOTIANA TABACUM. K. J. Jones and H. D. Shew, Department of Plant Pathology, NCSU, Raleigh NC 27696-7616.

The effect of resistance of four cultivars of N. tabacum on above ground symptom (AGS) expression and root infection was quantified in the field at two sites for 2 yr. Seedlings were grown using standard cultural practices for 40 days. Inoculations were made by placing 25ml of naturally-infested soil followed by 100ml of water at 7.5 or 15 cm from the stem and 7.5 or 15 cm deep in the soil. The raised row was then covered with polyethylene to prevent flooding during rainfall. Susceptible cultivars had higher levels of AGS than more resistant cultivars. Inoculation close to the stem resulted in more rapid and greater development of AGS across all cultivars. Shallow inoculation of adventitious roots that arose from buried stems resulted in more rapid and greater development of AGS than deep inoculation of true roots. The more resistant cultivars had a lower percent infection and a lower percentage of the infections resulted in AGS.

A566

MOVEMENT OF GENETICALLY MODIFIED RHIZOSPHERE BACTERIA (P. AUREOFACIENS) INTO THE AERIAL PORTION OF CORN PLANTS. T. G. Lamb, D. A. Kluepfel, and D. W. Tonkyn, Clemson University, Dept. of Bio. Sci., Clemson S. C., 96934.

The movement of genetically modified bacteria from the site of application presents a risk to non-targeted organisms. To determine the extent of this movement, corn seeds were inoculated with a lac ZY modified rhizosphere inhabiting bacteria, Pseudomonas aureofaciens (L-11), and the stems, leaves, and guttation drops were tested for 21 days. L-11 attained its maximum population size (1.1×10^5 CFU / g fresh wt.) in the stem 4 days after inoculation and then declined to its minimum at day 21 (1.9×10^3 CFU / g) . However, the population of L-11 in the leaves declined from a maximum (1.9×10^3 CFU / g) on day 5 to being detectable in only 42% of the plants in subsequent samples. L-11 was detectable in the guttation drops (5.5×10^3 CFU / ml) from the emergence of the corn shoot untill day 6. The major source of inoculum for the foliar tissue was the contamination of the shoot as it moved through the soil and the subsequent ingress of the bacteria into the interior through stomata and hydathodes.

A567

SURVIVAL OF BINUCLEATE RHIZOCTONIA FUNGI IN FIELD SOIL AND BEAN STEMS UNDER FIELD CONDITIONS. **M.A. Cubeta and E. Echandi, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695.**

Survival of binucleate Rhizoctonia fungi [CG232 and J3N1, (AGA)] in soil and precolonized bean stems was studied. CG232 and J3N1 were buried at 1, 15 and 30 cm deep in Clayton (CL) and Raleigh (RA) soils and sampled monthly for 1 yr. Populations of CG232 and J3N1 increased 73 and 40%, respectively, 1 month (mo.) after burial at 1 cm in RA soil. Neither isolate was recovered 4 mo. after burial at 30 cm or 7 mo. after burial at 1 and 15 cm in RA soil. In CL soil, CG232 and J3N1 increased 29 and 54%, respectively, 1 mo. after burial at 1 cm. After 9 mo. in CL soil, neither isolate was detected at 1, 15, or 30 cm. In precolonized bean stems, CG232 and J3N1 decreased by 50% 1 mo. after burial at 1 and 15 cm in CL and RA soil, but 3-5 mo. later, increased 10-30%. Neither isolate was recovered from colonized bean stems 11 mo. after burial in CL and RA soils. Populations of both isolates initially increased after 1 mo. in soil, but did not survive more than 9 mo. in soil and 11 mo. in bean stems.

A568

EFFECT OF SOIL pH ON GERMINABILITY AND VIRULENCE OF, AND LOSS OF ENDOGENOUS CARBON FROM, COCHLIOBOLUS SATIVUS CONIDIA. M. Hyakumachi[1], N. Suzuki[1], H. Ikegami[1], and J. L. Lockwood[2]. [1]Gifu University, Gifu 501-11, Japan and [2]Department of Botany & Plant Pathology, Michigan State University, East Lansing, MI 48824.

Cochliobolus sativus conidia were exposed for 30 days to three different soils adjusted with H_2SO_4, $(NH_4)_2SO_4$, KOH or K_2CO_3 to achieve a range of pH's from 5-9. Conidial germination was not detected on soils regardless of pH, but germination occurred when soils were supplemented with glucose. A larger amount of glucose was required to obtain equal germination rates on soils of higher than of lower pH. The ability of conidia to germinate in a salts solution without an exogenous energy source was rapidly lost during exposure to soils of higher pH. Virulence also declined more rapidly on soils of higher pH. Conidia lost more exogenous carbon during exposure of ^{14}C-labelled conidia to soil at pH 9 than at pH 5. Thus, soils with a high pH were more fungistatic and imposed a greater energy stress on conidia than soils with a lower pH.

A569

SUPPRESSIVENESS AND CONDUCIVENESS OF SOIL TO RHIZOCTONIA SOLANI AFTER CONSECUTIVE CROPPING. M. E. de la Fuente and C. A. Martinson. Iowa State University, Dept. Plant Pathology. Ames, IA, 50011.

Soil was infested or not infested with R. solani AG-4, planted with radish (host), cucumber (host), wheat (nonhost), or not planted, and then replanted (or not) for six cycles. One unplanted treatment was reinfested with R. solani each cycle. Then all soils were infested with R. solani and planted to radish. All soils became highly suppressive to R. solani except the soil never cropped and not infested originally. Soil conduciveness was measured by the linear development of disease in radish planted radially around a R. solani pellet, and by weighing the soil aggregate formed by its hyphae radiating from the pellet after 8 days. The latter procedure was the most definitive. Adding R. solani inoculum to the soil each cycle with no cropping resulted in the least conducive soil; the most conducive one was never cropped nor infested with the pathogen. Infestation with R. solani resulted in less conduciveness than with no infestation, and conduciveness was greater with cucumber and wheat cropping and less with radish cropping.

A570

GENOTYPIC REACTION OF WHEAT TO INFECTION BY PYTHIUM ARRHENOMANES, OR EXPOSURE TO ITS TOXIC METABOLITES. H. Mojdehi, and L. L. Singleton. Dept. of Plant Pathology, Oklahoma State University, Stillwater, OK 74078.

Seven wheat genotypes, some of which were earlier reported to be resistant to P. arrhenomanes (PA), and the variety TAM-101 were inoculated by placing 2-day-old seedlings on the edge of a PA culture for 3 h. Infected seedlings were transferred into test tubes (18 mm dia.) with glass beads and 1 ml of sterile water, and incubated for 4 days at 25 C. For toxic metabolite study, non-inoculated seedlings were placed in test tubes with 1 ml of a 25-day-old culture filtrate of PA. In all genotypes, significant reduction in root, and shoot growth was obtained by infection, or exposure to toxic metabolites. However, TAM-101 had significantly longer root length, and greater fresh and dry root weights than all other genotypes except one. Root or shoot growth of TAM-101 was not different from most other genotypes after exposure to toxic metabolites.

A571

PECTIC ZYMOGRAM CHARACTERISATION GIVES MORE INFORMATION THAN ANASTOMOSIS GROUPING OF STRAINS OF RHIZOCTONIA SOLANI WITHIN BARE PATCHES OF CEREALS IN WESTERN AUSTRALIA. G. C. Mac Nish and M. W. Sweetingham, Department of Agriculture, Esperance, 6450, Australia.

The Rhizoctonia spp. in a large rhizoctonia bare patch that stretched across four plots of alternating wheat and barley was studied. Wheat seedlings were grown in undisturbed soil cores removed from the patch over a 12 month period. R. spp. were isolated from the wheat roots and characterised using electrophoresis in pectin acrylamide gels. The zymogram patterns demonstrated that the patch was in fact a coalescing of two patches dominated by two strains (ZG1-1 or ZG2). These strains appeared not to mix, but maintained a distinct (but invisible to the naked eye) demarcation over the 12 month period. As both ZG1-1 and ZG2 belong to the same anastomosis group (AG8), the use of the latter method of identification would have failed to demonstrate the above phenomenon. Pathogenicity tests have shown that the virulence of ZG1-1 and ZG2 is similar on wheat but differs on lupin.

A572

FUSARIUM ON SPRING WHEAT UNDER CONVENTIONAL AND REDUCED TILLAGE. B. Salas and R. W. Stack. Dept. Plant Pathology, North Dakota State University, Fargo, ND 58105.

The influence of conventional and reduced tillage on *Fusarium* infection of spring wheat was studied by quantitative isolation from roots and crowns of plants grown in field trials. Of twenty-two *Fusarium* species identified, just six accounted for over 90% of the 5500 cultures isolated in 1987-1989. *F. equiseti* was the most frequently recovered (33.4 %) and was the only species which was found more often on plants grown under conventional tillage. The cereal root rot pathogens *F. avenaceum, F. culmorum* and *F. graminearum* together accounted for 21.9 % of cultures. Each of these as well as *F. acuminatum* was isolated at twice the frequency from plants growing under reduced tillage as under from those under conventional tillage. Isolation of *F. oxysporum*, the second most common species, was not affected by tillage.

A573

EFFECT OF BLACK PLASTIC AND NITROGEN ON DISEASE AND YIELD OF EGGPLANTS INFECTED WITH VERTICILLIUM DAHLIAE. W. H. Elmer and F. J. Ferrandino, Dept. of Plant Pathology and Ecology, The Connecticut Agricultural Experiment Station, Box 1106, New Haven, Connecticut 06504.

Eggplants were grown in two fields that differed in initial inoculum densities of Verticillium dahliae. A 2 X 2 factorial design of black plastic (P) or bare ground with or without a nitrogen sidedress (100 kg N/ha) at 45 days after planting (DAP) was studied for disease and yield response. Four replicated treatments were blocked twice in each field. Disease incidence, severity and yield were recorded during the season. At final harvest (DAP 95), plants were weighed, and stem and root colonization by V. dahliae was determined by placing tissue on selective media. The P + N, and P treatments increased yields, but did not affect incidence, severity or host colonization. Inasmuch as these treatments did not reduce symptom expression or host colonization, their ability to increase yield was apparently through increasing plant tolerance.

A574

COMPUTER ASSISTED STUDIES OF SOIL-BORNE FUNGI IN THE RHIZOSPHERE. H. T. Wilkinson and W. L. Pedersen. Dept. of Plant Pathology, Univ. of Illinois, Urbana, IL 61801.

A program designed to teach students about root pathogenesis was developed using wheat and the soil-borne pathogens, Geaumannomyces graminis var. tritici and Magnaporthe poae. The study program integrates a literature review, a lecture, a wet laboratory experiment, analysis of data, and the generation of a rhizosphere model. Data generated in the lab experiment are added to an existing data base, which is expanded by each group of experimenters. The computer software presents a menu driven program that the student can use to investigate the behavior of these pathogens in the rhizosphere. The student will select any combination of parameters listed and the computer calculates the maximum estimated distance from the root that a propagule can produce an infection. The program also will construct this rhizosphere cylinder in both two and three dimensions. The program is written in "C", a computer language, and operates in the Microsoft Windows environment.

A576

INFLUENCE OF TWO SOYBEAN CULTIVARS ON SOIL POPULATION DENSITIES OF MACROPHOMINA PHASEOLINA. S.R. Kendig and J.C. Rupe, University of Arkansas, Fayetteville, Arkansas 72701.

Soils were assayed for microsclerotia (ms) of Macrophomina phaseolina during the 1988 growing season. The cultivars, Davis and Lloyd, were planted and grown under the various irrigation regimes: no irrigation, full-season, until flowering, and after flowering. Ms soil densities decreased during the growing season with no differences between irrigation regime or cultivar. Although preplant populations the following year were not influenced by irrigation, ms densities were significantly higher in plots planted to Davis (92 ms/ 3 g of soil) than those planted to Lloyd (82 ms/ 3 gram of soil). Data indicate soybean cultivars did not influence ms soil densities during the growing season, but the cultivar Davis did contribute to a greater population of M phaseolina for the following growing season.

A577

GERMINATION OF PHYTOPHTHORA CAPSICI OOSPORES IN VITRO. M. J. Hord and J. B. Ristaino, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Oospores were obtained by crossing opposite mating types of the heterothallic fungus Phytophthora capsici on clarified V8 juice agar and incubating in the dark at 24 C for approximately 60 days. Oospores were collected by centrifugation and treated with enzyme to remove mycelium. A droplet of oospore suspension was placed in sterile distilled water (SDW), root extract or soil extract in petri plates. Germination was microscopically examined directly in the petri plates. Oospores germinated between 16 and 32 C with maximum germination at 24 C. Germination of oospores produced in the dark was 18%, while germination of oospores produced in the light was 2%. Germination after 12 days incubation in SDW, soil extract and root extract was 24, 36 and 39%, respectively. Oospores incubated in soil extract produced germ tubes with sporangia, while those incubated in root extract germinated to form long germ tubes with small or no sporangia.

A578

THE INFLUENCE OF SOIL pH ON THE ECOLOGY OF P. aphanidermatum AND P. ultimum. Frank N. Martin and C.R. Semer, Plant Pathology Department, University of Florida, Gainesville, Fla. 32611.

The influence of soil pH on the ecology of P. aphanidermatum and P. ultimum was investigated in autoclaved and natural field soils using a model system which evaluated the saprophytic activity of the isolates. Soil pH was adjusted from 3.5 to 8.5 and allowed to equilibrate for 10 days prior to use. All tests were conducted with soil maintained at -0.1 bars. The saprophytic activity of P. aphanidermatum was minimal at pH 3.5, increased to maximum at pH 5.5 to 6.5 and decreased slightly thereafter. Results were similar for P. ultimum with the exception that there was a significant decrease in saprophytic activity as the soil pH was increased from pH 7.5 to pH 8.5. Both species behaved similarly in autoclaved and natural field soils, indicating that the influence of soil pH was a direct effect on the fungus and not an indirect effect mediated by other soil mircoflora.

A579

COMPARISON OF AN INDUCTIVE-REASONING EXPERT SYSTEM TO A NEURAL-NETWORK: APPLICATIONS TO PEST RISK ASSESSMENT. M. H. Royer and C. E. Miller, U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Planning and Risk Analysis Systems, 6505 Belcrest Rd., Federal Bldg., Rm 817, Hyattsville, MD 20782.

Two software shells were used to demonstrate how an inductive reasoning expert system and a neural network could be used to build a knowledge base and to identify logic in pest risk assessments (PRA). PRA is defined here as the scientific estimation of the likelihood of introduction and magnitude of the effects of establishment of an exotic pest. An example from USDA-APHIS-PPQ was used to demonstrate how PRAs could be performed for certain insects. Both shells were developed by formulating questions that mimicked the original assessment logic as much as possible. An expert familiar with the data was involved in the creation of the knowledge base. The neural network was the preferred tool of the expert, but may not be directly applicable for a regulatory agency due to the "lack of transparency" of the computer program. Applications in PRA for plant pathogens are suggested.

A580

SPATIAL DISTRIBUTIONS OF PSEUDOMONAS SYRINGAE STRAINS ON POTATO LEAVES. L. L. Kinkel and S. E. Lindow, Department of Plant Pathology, University of California, Berkeley, CA 94720.

The spatial relationships of bacteria coexisting on leaves are not well described. We investigated the spatial patterns of Pseudomonas syringae strains inoculated singly or in pairs onto potato leaves. Plants were maintained under alternating wet and dry conditions, and leaf samples were taken 24-72 h after inoculation. Entire leaves were sectioned into 0.09 cm^2 quadrats and population sizes of individual bacterial strains were estimated for each quadrat. Population sizes on single quadrats ranged from 0 to > 1500 individuals. Bacteria were aggregated on the leaf surface (variance/mean >> 1). Quadrats located along the central vein tended to have the largest population sizes. There were no apparent differences in the spatial distribution of strains when inoculated onto leaves singly or in pairs. More sophisticated approaches to describing the spatial patterns of bacteria on leaves in relation to specific microsites will be suggested.

A581

SAMPLING PHYLLOPLANE POPULATIONS: DISTRIBUTIONAL EFFECTS ON SAMPLE DESIGN. L. L. Kinkel, M. Wilson, and S. E. Lindow, Department of Plant Pathology, University of California, Berkeley, CA 94720.

We investigated the distribution of Pseudomonas syringae population sizes on individual leaves (n = 100) over time in growth chamber experiments. Population sizes among leaves were distributed lognormally immediately following inoculation and following the incubation of plants under both wet (bacterial growth) and dry (death) conditions. Variance was lowest in the sample immediately following inoculation, and varied from 0.081 to 0.309 in remaining samples (log cfu/g). Power curves were constructed for different hypothetical sample sizes based on observed variances among leaves and for bulked leaf samples created by combining data from random subsets of leaves. We used this information to determine appropriate sample sizes for desired power levels (given an expected variance). Though bulked leaf samples increase the amount of material sampled and can reduce experimental variance, they may not provide a biologically useful variance estimate.

A582

SURVIVAL AND SPREAD OF CLAVIBACTER MICHIGANESIS SUBSP. MICHIGANENSIS IN TOMATOES. M. L. Gleason, E. J. Braun, W. M. Carlton, Department of Plant Pathology, Iowa State University, Ames, IA 50011.

Rifampicin-resistant strains of Clavibacter michiganensis subsp. michiganensis (Cmm), the causal agent of bacterial canker of tomato, were used to investigate epiphytic survival, over-wintering, and vascular colonization by the pathogen. Epiphytic populations stabilized at 10**6 to 10**8 cfu/leaflet and 10**5 to 10**6 cfu/green fruit. Cmm survived the winter in infested debris, but survival on the soil surface was much greater than in buried debris. Overwintered, infested debris significantly reduced yield in a subsequent processing tomato crop. Cmm spread more rapidly through the vascular system of 17-day-old seedlings than of 45-day-old seedlings. Vascular colonization from epiphytic populations was also accelerated by removal of axillary leaves of fresh market tomatoes. The findings suggest that epiphytic populations of Cmm can be significant in the disease cycle of canker.

A583

PRESENCE OF *ERWINIA CHRYSANTHEMI*, CAUSAL AGENT OF STEM AND ROOT ROT, ON SWEETPOTATO THROUGH THE GROWING SEASON. **V. Duarte, and C. A. Clark. Dept. Plant Pathology & Crop Physiology, Louisiana Agric. Expt. Sta., LSU Agric. Center, Baton Rouge 70803-1720.**

In 1988 and 1989, storage roots of cv. Beauregard were inoculated at bedding by either dipping them without injuring in a suspension of a rifampicin-resistant, virulent strain of *E. chrysanthemi* (Ech-2-rr) or by inserting a pipette tip containning 50 µl of the same suspension into the root. At transplanting, slips were cut or pulled and transplanted to the field two times in succession. Ech-2-rr was recovered from inoculated roots at 0 and 7 days after bedding, and at the 1st and 2nd pullings. The bacterium was also present on symptomless vine cuttings after transplanting to the field, on the below-ground stem region, and on daughter storage roots at harvest. However, despite high rainfall and temperature, particularly in 1989, little stem rot was observed in the field. Inoculation of vine cuttings at transplanting did not affect disease occurrence. There was no difference in pathogen or disease occurrence when pulled or cut slips were used for transplanting. When cv. Jewel slips were cut with a contaminated knife, Ech-2-rr was recovered from the cut stub of the sprout, from transplants at the subsequent cutting, from the surface of the mother root, and from the below-ground stem region at harvest.

A584

Evaluation of the Iowa pod test for foliar fungicides on soybeans in Kansas. Douglas J. Jardine, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502.

The Iowa pod test to determine the need for foliar fungicide sprays in improving seed quality was tested in Kansas. Pods were collected and evaluated for presence of Phomopsis at soybean growth stage R6. In 1987-1989, percent pod infection was 50, 14, and 50, respectively. Accordingly, spraying would have been required in 1987 and 1989. After harvest, seeds were evaluated for the presence of seed-borne pathogens. In 1987 and 1988, there were no significant differences in the level of pathogens present on seeds from the untreated or benomyl sprayed plants. In 1989, Penicillium was significantly reduced on seeds from benomyl sprayed plants, but Alternaria and Fusarium significantly increased. Fungicide treatment did not improve germination in 1987 and 1989 when a fungicide treatment was called for. Germination percentage significantly increased in 1988 when no spray was predicted. The model did not reliably predict the need for foliar fungicides in Kansas.

A585

ASCOCARP PRODUCTION BY PYRENOPHORA TRITICI-REPENTIS IN WHEAT STRAW UNDER INTERMITTENT WETTING. W. Zhang, and W. Pfender. Dept. Plant Pathology, Kansas State Univ., Manhattan, KS 66506.

The effect of wetting duration on ascocarp production by Pyrenophora tritici-repentis (Ptr) in wheat straw was studied using two types of straw: axenic straw (sterilized, then recolonized with Ptr) and field straw (field-grown, naturally-infested with Ptr and saprophytic microorganisms). Both types of straw were exposed to three regimes of alternating moisture and desiccation, in which the repeated wetting period was 5, 10, or 36 hr, respectively. Ascocarps developed in all 3 wetting treatments. However, in the 36-hr treatment, ascocarps formed earlier than in the 5-hr treatment (2 and 4-10 wetting cycles earlier for small and large ascocarps, respectively); the 10-hr treatment was intermediate. Ascocarp production in field straw was more variable than that in axenic straw, and differences between 36-hr and 5-hr treatments were accentuated. Ascus formation was correlated with wetting-cycle duration. The relationship of these data to field observations of straw wetness duration will be discussed.

A586

INFLUENCE OF SURFACE CORN RESIDUES ON DISEASE GRADIENTS OF GRAY LEAF SPOT OF CORN. N. R. X. de Nazareno, P. E. Lipps and L. V. Madden, Dept. of Plant Pathology, The Ohio State University and Ohio Agricultural Res. and Dev. Center, Wooster, OH 44691.

Spread of corn gray leaf spot (GLS), caused by Cercospora zeae-maydis Teh. and Dan., was studied at Dresden and Columbus, Ohio. Dresden had a history of GLS epidemics and Columbus had no previous record of GLS. Square blocks (930 m^2) of two hybrids (Pioneer Brand 3352 and LH119 x LH51) were planted on May, 1989. Corn residue was collected from the surface of a field that had GLS the previous growing season and spread on the surface of a 9.3 m^2 area in the center of each block when plants were at fifth leaf stage. Disease spread was assessed weekly from appearance of first lesions within and across corn rows in four directions from the edge of the residue. At Columbus a steep disease gradient was observed extending from the inoculum source out 3 m, with more pronounced spread down than across rows. However, at Dresden, no definite disease gradient was detected, presumably due to the presence of other sources of inoculum in addition to the infested corn residue placed in the center of the blocks.

A587

EFFECT OF SURFACE TOPOGRAPHY AND RAIN INTENSITY ON RAIN SPLASH DISPERSAL OF COLLETOTRICHUM ACUTATUM OF STRAWBERRY. X. Yang, L. V. Madden, L. L. Wilson, and M. A. Ellis, Dept of Plant Pathology, Ohio State Univ., Wooster, OH 44691

Effects of ground cover (straw, soil, and plastic), leaf area index (0, 2.7, and 4.9) of a strawberry row canopy, and rain intensity (15 and 30 mm/hr) on rain splash dispersal of Colletotrichum acutatum were studied, using a rain simulator. Dispersal was measured by collecting droplets containing conidia in gravity samplers positioned at various distances from the source at selected times. Ground cover had a major effect, as measured by colonies growing in a selective medium; straw had the fewest colonies, plastic the most, and soil intermediate. Differences in covers were due to differences in steepness of gradients (straw had the steepest and plastic the shallowest), not release rate from the source. Colonies increased with rain intensity. This was due to the release rate, as measured by the intercept parameter of a gradient model, not gradient steepness. Leaf area index was inversely related to colony number.

A588

Ascospore discharge of Anisogramma anomola under field and controlled conditions. J.N. Pinkerton[1], K.B. Johnson[2], J.K. Stone[2], and J.W. Pscheidt[2]. [1]USDA/ARS Hort. Crops Res. Lab., Corvallis, OR 97330, and [2]Dept. of Botany & Plant Pathology, Oregon St. Univ., Corvallis, 97331-2902.

Ascospores of A. anomola, the cause of eastern filbert blight, were trapped in a hazelnut orchard from 4 November 1988 to 1 June 1989. Rain collectors were placed under cankers in ten trees. Collector reservoirs were changed weekly and spore discharge per canker per week was calculated by counting spores within subsamples of the rain water. Precipitation was monitored on an hourly interval. Samples from November accounted for 75% of the total number of spores trapped. This month had 177 hr of precipitation. Spores were trapped in each week with rain from December to April. Few spores were trapped in May after a cumulative 660 hr of rain for the sampling period. In a growth chamber, spore discharge from cankers ceased after 720 hr of intermittent mist at 15 C. Additional growth chamber and field data were collected in 1989-1990.

A589

EFFECT OF ENDOPHYTE INFECTION OF PERENNIAL RYEGRASS ON GROWTH UNDER DROUGHT STRESS. M. L. Gleason, N. E. Christians, and M. Agnew, Departments of Plant Pathology and Horticulture, Iowa State University, Ames, IA 50011.

Reports that infection by a fungal endophyte, Acremonium loliae, improves tolerance of perennial ryegrass to drought stress were evaluated in greenhouse experiments. Endophyte was eliminated by growing tillers in sand amended with 200 ppm benomyl. Same-clonal plants with and without endophyte were grown in field soil amended with 5 g/L 14-14-14 fertilizer. Pots were weighed daily and watered to saturation when pot weights corresponded to soil water potentials of -0.4, -2.0, or -15 bars. After 7 wk in one trial, shoot and root dry weights of endophyte-free plants were significantly greater than for endophyte-infected plants at all watering treatments. In two other trials, endophyte infection had no significant effect on growth at any watering treatment.

A590

THE EFFECT OF TEMPERATURE ON ASCOSPORE RELEASE, GERMINATION AND INFECTION BY UNCINULA NECATOR ON GRAPE. C. S. Thomas, W. D. Gubler, and D. Fogle. Department of Plant Pathology, University of California, Davis, CA 95616.

Mature cleistothecia of Uncinula necator (Schw.) Burr. were collected from a commercial chardonnay vineyard (Vitis vinifera L.) in Monterey Co., California. The number of ascospores released was determined on water agar at seven temperatures from 5 to 35 C. Ascospore germination and infection were determined at five temperatures from 10 to 30 C on Carignane grape leaves. The greatest number of spores were released at 15 and 20 C. Of the ascospores that were released at each temperature, a significantly greater percentage germinated at 15 and 20 C. Infection occurred throughout the temperature range tested (10-30 C) but was greatly reduced at 30 C.

A591

EPIMODEL - A COMPUTER PROGRAM TO TEACH PRINCIPLES OF MODELLING PATHOGEN POPULATION GROWTH AND ANALYSIS. F. W. Nutter, Jr. and O. Worawitlikit. Department of Plant Pathology, University of Georgia, Athens, GA 30602.

EPIMODEL was developed for the purpose of teaching students principles of pathogen population growth modelling. Example data sets are available within the program to demonstrate the application of several growth models (monomolecular, exponential, logistic, gompertz). EPIMODEL can also be used to help students select the most appropriate growth model for temporal disease assessment data sets. The program is menu-driven and allows easy entry of actual data sets for study. The program will produce graphs of the untransformed data vs time, dy/dt (rate) vs time, the transformed data vs time, and the residuals vs time. Regression statistics and parameters are also displayed for each growth model and hard copies of all data and graphs can be produced.

A592

TIME-SEQUENCED INOCULATION INDICATES A SUSCEPTIBILITY RHYTHM IN SOYBEAN BUDBLIGHT DISEASE. B.W. Kennedy and R. Denny, Department of Plant Pathology, University of Minnesota, MN 55108.

Glycine max L. cv bansei were grown in controlled environments at constant 25 C, 85 ± 5 percent RH and a 12:12 h light (L) dark (D) regime in which 12 h darkness followed 12 h of light. One primary leaf of a subset of 7-day-old seedlings was inoculated every four h for 20 h with buffered extracts of fluid from soybean plants infected with tobacco streak virus. Leaf movements were recorded by measuring leaf angles every four h for 96 h and data were subjected to curve-fitting procedures to detect relationships between leaf movement and symptom development (epinasty of growing tips and inoculated leaves). Leaf angles from plants during the first LD cycle following inoculation (day 1) were subtracted from those in the second (day 2) and the second from the third. Amplitude of leaf angle differences from day 1 to day 2 and from day 2 to day 3, were statistically different via cosinor analysis; there were no differences in circadian timing of the change but differences within the first day after inoculation predicted symptoms to come.

A593

EFFECTIVE VESICULAR-ARBUSCULAR (VA) MYCORRHIZAL FUNGI FOR PUERARIA PHASEOLOIDES GROWN IN A HIGH-ALUMINUM ACID SOIL. H. T. Bartolome and N. C. Schenck, Plant Pathology Department, University of Florida, Gainesville, FL 32611.

Thirteen isolates from the International Culture Collection of VA Mycorrhizal Fungi (INVAM) were screened for effectiveness in improving the growth and nodulation of Pueraria phaseoloides

grown in a high-Al acid soil (pH 4.2; 308 mg/kg Al). Glomus manihot INVAM Isolate LMNH 980 (indigenous in the test soil) was the most effective in colonizing the root and improving the growth of Pueraria. Gigaspora gigantea GGGT 109 and 663, G. margarita GMRG 185 and 444, Entrophospora colombiana ECLB 356, and Scutellispora heterogama CHTG 139 also significantly improved growth and nodulation of the host. Scutellispora calospora CCLS 348 and S. pellucida CPLC 288 colonized Pueraria but did not significantly improve growth and nodulation of the latter. Glomus etunicatum LETC 236, G. mosseae LMSS 378, and Acaulospora scrobiculata ASCB 456 did not colonize Pueraria in the high-Al acid test soil. Thus, VA mycorrhizal fungi must be selected for soil edaphic factors such as acidity and Al levels.

A594

CHARACTERIZATION OF MYCORRHIZOPLANE-ASSOCIATED STREPTOMYCETE EFFECTS ON ECTOMYCORRHIZAL FUNGI. S.M. Paetschow, S.T. Bagley, and J.N. Bruhn. Michigan Technological University, Houghton, MI 49931

Up to 25 different streptomycete morphotypes were isolated by enrichment technique from the mycorrhizoplane of red pine seedlings during monthly sampling over a 3 year period. Morphotypes were screened for effects on three red pine ectomycorrhizal fungi, *Laccaria bicolor*, *L. laccata*, and *Thelephora terrestris*. Six morphotypes showed the desired pattern of promoting growth of *Laccaria* spp. but inhibiting *T. terrestris*. The effects of growth medium (MMN, nutrient, and yeast extract), temperature (25, 30, and 35°C), pH (5.5, 6.0, 6.5, 7.0, 7.5), and shaking vs. static incubation on biomass and fungal effects were determined. Fungal growth effects were assayed by incorporating sterile filtrate (0-60%) into MMN agar. Optimal conditions for streptomycete biomass and fungus-affecting compound production for all six morphotypes were obtained with MMN broth at pH 7.0 and 30°C with static incubation. Peak effects were seen with 50% filtrate. Studies are continuing with the two morphotypes causing the greatest responses to determine the type of compound(s) produced.

A595

COLONIZATION OF RESISTANT AND SUSCEPTIBLE CORKY ROOT LETTUCE CULTIVARS BY GLOMUS INTRARADICES. L.E. Datnoff, R.T. Nagata, Univ. of FL-EREC, Belle Glade and T.E. Wood, NPI, Salt Lake City, UT

Corky root caused by Rhizomonas suberifaciens is a disease of lettuce (Lactuca sativa L.) grown in south Florida. Vesicular-arbuscular mycorrhizae (VAM) could be used as a biocontrol agent and affect corky root development. VAM colonization of resistant (R) and susceptible (S) lettuce cultivars to corky root were investigated. 'Shawnee', S, and 'South Bay', R, crisp-head lettuce cultivars were inoculated with Nutri-Link, a VAM inoculant containing spores of G. intraradices. Seeds were planted in a potting medium non-amended and amended with 500 spores per cell. Plants were fertilized with a 20-2-20 liquid formulation. After 28 days, plants were observed for colonization and effects on dry weight. Generally, no differences were noted for percent colonization between cultivars or dry weight between colonized or non-colonized plants.

A596

RELATEDNESS STUDIES ON *PISOLITHUS TINCTORIUS* FROM NORTH AMERICA, ASIA, AND EUROPE. J.B. Szerszen, R.A. Taber, and R.E. Pettit, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas 77843-2132.

Isolates of the ectomycorrhizal gasteromycete *Pisolithus tinctorius* from 15 different geographical regions throughout the world were used in this study. The fungi were grown on synthetic medium at 22C for 21 days. Their buffer-soluble proteins were electrophoretically compared by microprocessor-controlled NATIVE-PAGE, SDS-PAGE, IEF-PAGE, and two-dimensional electrophoresis. Twenty five protein components were separated from the mycelia of the fungi in the presence of SDS (MW range 14,100-72,400 daltons) and 40 components using isoelectrofocusing (pI range 3.60-7.45). Isolates from Texas and Georgia had identical SDS and IEF electrophoretic patterns. The SDS and IEF patterns of the Philippine isolate were different than U.S. or European isolates; SDS gels revealed the Philippine isolate lacked 2 major and 2 minor polypeptides, and IEF revealed it lacked 14 components found in the U.S. isolates. Other isolates also showed differences in banding patterns. There were also differences among the isolates in activities and banding patterns of certain isozymes. General native protein, polypeptide, and isozyme patterns of all isolates investigated were similar, however slight differences in these patterns allowed to distinguish certain isolates.

A597

Virus-like Particles in *Plasmopara halstedii*, Sunflower Downy Mildew. T. J. Gulya, T. P. Freeman, and D. E. Mayhew. USDA Northern Crop Science Lab; NDSU Electron Microscope Lab, Fargo, North Dakota 58105; and Analysis & Identification Lab, CDFA, Sacramento, California 95814.

Virus-like particles (VLP) were observed in high-titer in hyphae, haustoria, and zoosporangia of *Plasmopara halstedii* race 2. The particles were icosohedral and measured 26 nm when stained with phosphotungstic acid in leaf dips or 24.3 nm in sectioned material. Four species of double stranded RNA with approximate molecular weights of 3.08, 2.05, 1.76 and 1.03 kd were found in sunflower tissue infected with *P. halstedii*. No ds-RNA was found in healthy sunflower tissue. This is the first report of VLPs in *Plasmopara* spp. All isolates of race 2 examined contained VLPs. Attempts at mechanical transmission using triturated zoosporangia failed to yield any symptoms on any indicator hosts.

A598

HOST SUSCEPTIBILITY AS THE BASIS FOR TRANSMISSION DIFFERENCES AMONG THREE BEETLE-TRANSMITTED VIRUSES. T. K. Field, H. A. Scott, and R. C. Gergerich. Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Three stable, single component viruses, southern bean mosaic (SBMV), bean mild mosaic (BMMV), and blackgram mottle (BGMV) were transmitted with different efficiencies by Mexican bean beetles using *Phaseolus vulgaris* 'Black Valentine' bean as the acquisition and test host (SBMV = 95%, BMMV = 41%, BGMV = 22%). When equal concentrations of purified viruses were inoculated by beetles or by gross wounding, SBMV = BMMV > BGMV. Translocation in cut stems and indirect immunofluorescent analysis of the three purified viruses in gross-wounded primary leaves demonstrated rapid movement in vascular tissue. Comparisons of viral infectivities using mechanical inoculation demonstrated that Black Valentine bean is less susceptible to BGMV. Furthermore, BGMV and BMMV concentrations are lower than SBMV in infected bean. Thus, reduced transmission rates result from lower test host susceptibility and virus titer in the acquisition host.

A599

BIOCHEMICAL ALTERATIONS ASSOCIATED WITH BEAN POD MOTTLE VIRUS (BPMV) PATHOGENESIS OF *PHASEOLUS VULGARIS* (BEAN) CV. PINTO. P. L. Popham, A. Novacky, and O. P. Sehgal. 108 Waters Hall, Dept. of Plant Pathology, University of Missouri, Columbia, Mo. 65211.

Active oxygen production, lipid peroxidation, electrolyte leakage, cell death, and necrosis are characteristics of a typical hypersensitive reaction (HR). BPMV does not induce necrotic lesions on Pinto, but an active host resistance mechanism that limits viral spread exists. Lesions caused by BPMV are in the form of discolorations of the minor vascular bundles in the infection area. Superoxide production has been demonstrated during BPMV pathogenesis of Pinto, but lipid peroxidation has not been detected. The percentage of dead to live cells is higher in BPMV-infected tissues than in the controls. In a typical viral induced HR, however, all cells within a necrotic lesion are dead. Electrophysiological experiments demonstrate that the electrical potential across the plasmalemma is reduced upon BPMV infection. The reaction of Pinto to BPMV infection does not completely parallel events involved in HR; however, some similarities exist.

A600

RELATIONSHIPS AMONG SEROTYPES OF COWPEA SEVERE MOSAIC VIRUS AS DETERMINED BY SIGNATURE ANALYSIS. R. Di, J. H. Hill, and R. A. Van Deusen, Dept. of Plant Pathology, Iowa State University, Ames, IA 50011.

Isolates of cowpea severe mosaic virus (CPSMV) can be grouped into nine serotypes (I to IX) by immunodiffusion tests using polyclonal antibodies (Lin et al., 1981, 1984). Several common and serotype-specific antigenic determinants have been identified. In this study, the nine serotypes were compared using "signature analysis". Radioimmunoassays, using seven monoclonal antibodies (MAbs) that recognize CPSMV serotype-specific or cross reactive epitopes, were used to construct antigenic signatures over a range of antigen concentrations. A statistical (iterative least-squares) method was used to align unknown CPSMV antigen concentrations from different virus preparations to allow comparison of binding profiles from different assays. The technique could identify antigenic divergence among different serotypes of the virus.

A601

FRAMESHIFT IN TRANSLATION OF BYDV RNA. R. Di, W. A. Miller, Dept. of Plant Pathology, Iowa State University, Ames, IA 50011.

Based on the genome organization of BYDV, the polymerase gene (60K ORF) was proposed to be translated via a frameshift event

in the overlapping region of the 39K and 60K ORFs. In vitro translation of BYDV (PAV serotype) genomic RNA gives products consistent with translational frameshifting, a major band migrating at 39K accompanied by a minor band of 98K (about 39K + 60K). The frameshifting sequence and structure of BYDV RNA (PAV serotype) have been tested in vivo. Sequences with different lengths have been inserted into the LacZ reporter gene in E. coli. The translational frameshifting event has been demonstrated as measured by the enzymatic activity of β-galactosidase.

A602

DEVELOPMENT OF CYLINDRICAL INCLUSIONS IN POTYVIRUS-INFECTED PROTOPLASTS. J.F. Murphy, U. Jarlfors and J.G. Shaw, University of Kentucky, Lexington, KY. 40546.

Distinctive aggregates of potyviral cylindrical inclusion protein (CI) molecules are found in the cytoplasm of infected cells. There have been many reports of the development of cylindrical inclusions in cells of infected leaf tissue, but none to our knowledge of their development in isolated protoplasts. Tobacco mesophyll protoplasts were inoculated with tobacco vein mottling potyvirus RNA by electroporation and collected at various times p.i. Cylindrical inclusions in the form of bundles and pinwheels were observed in protoplast sections as early as 15 hr p.i. When sections were subjected to immunogold labeling using anti-CI serum and gold-conjugated Protein A, cylindrical inclusions were detected as early as 10 hr p.i. Cylindrical inclusions were either associated with the plasma membrane or observed in the cytoplasm (with no apparent membrane association). There was an increase in the number of cylindrical inclusions over time (15-30 hr p.i.). The rate of increase in the number of cytoplasm-associated cylindrical inclusions appeared to be greater than that observed for cylindrical inclusions associated with the plasma membrane.

A603

REPLICATION OF SONCHUS YELLOW NET VIRUS IN PROTOPLASTS. R.W. Jones and A. O. Jackson, Department of Plant Pathology, University of California, Berkeley, CA, 94720.

Host tobacco protoplasts were infected with sonchus yellow net virus (SYNV), a plant rhabdovirus, and analyzed for viral protein and RNA synthesis. Transcription of SYNV mRNA was detected within two hr post-inoculation (PI), reached maximal levels by 24 hr and declined to undetectable levels by 60 hr. Replication of the negative-stranded genomic RNA was evident between 24 and 36 hr, but decreased appreciably by 60 hr PI. Synthesis of the four major viral structural proteins were detected by western analyses within 24 hr PI and these proteins achieved maximal accumulation by 43 hr PI. Association of viral protein accumulation with a switch from a transcriptional to replicative phase parallels some animal rhabdovirus systems. Among various glycosylation inhibitors applied to protoplasts, only tunicamycin effected viral protein synthesis, resulting in synthesis of a G protein about 10% smaller than in untreated protoplasts. Two specific cleavage products of the nucleocapsid (N) protein with molecular weights of about 21,000 and 37,000 appeared in cells by 60 hr PI, but in the presence of tunicamycin, the cleavage products were present by 38 hr PI. It may be possible that this specific cleavage of the N protein, activated in cells stressed by viral accumulation and/or tunicamycin treatment, accounts for the loss of virions and nucleocapsids during the chronic stage of SYNV plant infections.

A604

DETECTION AND DIFFERENTIATION OF POTYVIRUSES USING VIRUS-SPECIFIC AND BROAD SPECTRUM MONOCLONAL ANTIBODIES. Ramon Jordan and John Hammond, USDA-ARS, Beltsville, Maryland.

Bean yellow mosaic virus (BYMV)-specific, BYMV subgroup-specific and potyvirus group cross-reactive monoclonal antibodies (McAbs) were evaluated with respect to their ability to detect and differentiate potyviruses. Different extraction buffers, antigen preparation regimes, solid phase coating conditions and ELISA and dot-blot assay formats were tested. All McAbs detected viral antigens in antigen-coated plate (ACP) ELISAs. Virus-specific McAbs could also detect virus in triple-antibody-sandwich assays. All of the tested potyviruses were readily detected in ACP-ELISA by at least one McAb (PTY 1) with substrate incubation times ranging from 1 to 7 hr; however, some low virus titer-containing plant extracts required overnight substrate incubation for reliable detection. BYMV isolates from gladiolus, iris, orchid and pea, as well as most of the other distinct potyviruses tested, could be differentiated with the panel of McAbs. Individual or specific admixtures of McAbs were capable of identifying certain virus clusters or related subgroups.

A605

USE OF MONOCLONAL ANTIBODIES FOR DETECTION OF CYMBIDIUM MOSAIC POTEXVIRUS IN INFECTED ORCHIDS. H. T. Hsu, D. Vongsasitorn, and R. H. Lawson, USDA-ARS, Beltsville, MD 20705.

Mouse hybridomas secreting monoclonal antibodies to cymbidium mosaic potexvirus (CyMV) were produced by fusing immune splenocytes with FOX NY myeloma cells. Forty five CyMV monoclonal antibodies reacted with the homologous antigen trapped by rabbit anti-CyMV serum coated ELISA plates; twenty-nine of the 45 antibodies reacted with CyMV on antigen coated ELISA plates. In IEM on monoclonal antibody-coated grids the dilution end point of crude sap from infected orchids was about 1/800; while the end point of the same sap with the same monoclonal antibodies was about 1/400 in ELISA and 1/3200 by dot-blot assay on nitrocellulose membranes. An indirect immunological method was developed to detect CyMV antigens in direct tissue blots on nitrocellulose membranes. CyMV was detected in 30 of 155 plants in tissue blots on nitrocellulose membranes. Two and 4 samples out of the 30 that were positive in tissue blots showed A_{405} values less than 0.05 and 0.2, respectively, when tested at 1/20 sap dilutions.

A606

REACTIVITY OF TWO MONOCLONAL ANTIBODIES TO THE CYLINDRICAL INCLUSION PROTEIN OF PAPAYA RINGSPOT VIRUS TYPE-W. C.A. Baker and D.E. Purcifull. University of Florida. Gainesville, FL 32611

Spleen cells of BALB/c mice injected with SDS-PAGE purified cylindrical inclusion protein (CIP) from an isolate of papaya ringspot virus type-W (PRSV-W) were fused with Sp2/O myeloma cells. Two resulting monoclonal antibodies (MCA) reacted with the CIP band (MW = 68-70k) when crude sap from PRSV-infected tissue was analyzed by Western blotting. MCA CI-1 reacted with 15 PRSV-W isolates and with an isolate of papaya ringspot virus type-P (PRSV-P). MCA CI-2 reacted with 12 of 15 PRSV-W isolates, PRSV-P and the Tigre isolate of PRSV. Neither MCA reacted with the Moroccan isolate of watermelon mosaic virus (WMV-M), watermelon mosaic virus-2 (WMV-2), zucchini yellow mosaic virus (ZYMV) or healthy pumpkin. Polyclonal rabbit antiserum (PCA) to the CIP reacted with all isolates of PRSV tested, and cross-reacted with WMV-M, WMV-2 and ZYMV. The differential reactions of the two MCAs and the PCA indicate that the CIP has both specific and common epitopes which could be useful in the classification and diagnosis of potyviruses.

A608

EXPRESSION OF RESISTANCE OF HARD RED WINTER WHEAT TO WHEAT SOILBORNE MOSAIC VIRUS. J. L. Sherwood, L. D. Myers, and R. M. Hunger. Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947.

Although cultivars (cvs) of hard red winter wheat (Triticum aestivum L.) with resistance to WSBMV have been developed, the mechanism(s) of resistance is not understood. Larsen, et al. (Plant Disease 69:857) proposed that resistance to WSBMV is expressed as resistance to Polymyxa graminis carrying WSBMV or a reduced movement of WSBMV within roots of cvs resistant to WSBMV. Two susceptible cvs (Sage and Vona) and two resistant cvs (Hawk and Newton) were inoculated using root washings from WSBMV infected wheat (Plant Disease 69:848) or were mechanically inoculated. ELISA was used to detect WSBMV after inoculation. WSBMV was found in the shoots and roots of susceptible cvs regardless of the inoculation method. WSBMV was found in the shoots and roots of resistant cvs when mechanically inoculated, but only in the roots when plants were inoculated with root washings. An inhibition of virus movement is suggested as the mechanism of resistance.

A609

CHARACTERIZATION OF CITRUS RINGSPOT VIRUS. K. S. Derrick, R. F. Lee, B. G. Hewitt, G. A. Barthe, and J. V. da Graca. University of Florida, Institute of Food and Agricultural Sciences, Citrus Research and Education Center, Lake Alfred 33850.

The virus associated with citrus ringspot (CRSV) was further characterized. Nucleic acids associated with the short and long filamentous particles were isolated and appeared to be single-stranded RNA. Nucleic acid preparations from crude extracts or partially purified preparations were not infectious. A sedimentable double-stranded RNA was associated with CRSV infections. An antiserum to purified CRSV was used to detect virus particles blotted onto nitrocellulose from agarose gels and by serologically specific electron microscopy. The antiserum was also used to detect the 48 kilodaton protein associated with CRSV in western blots.

A610

ULTRASTRUCTURE OF MAIZE LEAVES SINGLY OR DOUBLY INFECTED WITH MAIZE CHLOROTIC MOTTLE (MCMV) AND MAIZE DWARF MOSAIC (MDMV) VIRUSES. E. D. Ammar[1] and D. T. Gordon[2], [1]Dept. of Entomology, Fac. Agric., Cairo Univ., Egypt; [2]Dept. of Plant Pathology, Ohio State Univ., Wooster, OH 44691

Maize seedlings mechanically inoculated with MDMV (strain B), MCMV, or both were processed for electron microscopy 2 or 3 wk post-inoculation. Particles and inclusions of MDMV were abundant in leaves of singly or doubly infected plants at both intervals; those of MCMV were abundant only at 3 wk in singly infected plants, and at both intervals in doubly infected ones. Previously undescribed 'complex' inclusions containing apparently deformed, enlarged or coalesced mitochondria, large aggregates of fibrous structures, MCMV and MDMV particles and inclusions, were abundantly found in doubly infected plants. Complex inclusions, without MDMV particles or its inclusions, were occasionally found in MCMV-singly infected plants. The significance of these ultrastructural alterations in the development of the corn lethal necrosis disease is discussed.

A611

DETECTION OF CITRUS TRISTEZA VIRUS (CTV) WITH A MIXTURE OF MONOCLONAL ANTIBODIES. M. Cambra, I.V.I.A., Moncada, Spain; S. M. Garnsey, T. A. Permar, C. T. Henderson, USDA, ARS, Orlando, FL; D. Gumpf, University of California, Riverside; and C. Vela, Ingenasa, Madrid, Spain.

Although several epitopes of CTV are well conserved, none of the existing monoclonal antibodies (Mabs) react with all CTV isolates. Eighty-four isolates of CTV from 18 countries (selected for maximum serological and biological diversity) were tested with a mixture of two Mabs (IgG2b) specific to different epitopes and against polyclonal antisera in four variations of enzyme-linked immunosorbent assay (ELISA). The Mab mixture did react to all isolates tested and proved a suitable substitute for polyclonal antisera in double antibody sandwich ELISA. The best results were obtained with a biotin-strepavidin protocol using unlabeled and biotinylated Mab mixture to trap and detect CTV antigens.

A612

CROSS PROTECTION WITH TOBACCO MOSAIC VIRUS IN ARABIDOPSIS THALIANA. L. A. Urban, J. L. Sherwood, and U. K. Melcher. Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695; Department of Plant Pathology, and Department of Biochemistry, Oklahoma State University, Stillwater, OK 74078-9947.

To test the role of tobacco mosaic virus (TMV) coat protein in cross protection in A. thaliana, three week old plants were initially inoculated with 50 ug/ml of TMV isolated from petunia (TMV-P, Virology 119:150). Plants were challenged with 10 ug/ml RNA from the common strain of TMV (TMV-C) 7 days later. After 10 days, the presence of coat protein of each strain was determined in the initially inoculated leaf, the leaf that was challenge inoculated, and a leaf above the challenge inoculated leaf. TMV-P was detected throughout the plants. TMV-C was rarely detected out of the leaf onto which it was inoculated. Similar results were obtained when virions of TMV-C were used as the challenge inoculum. These results suggest that A. thaliana inhibition of movement of the challenge virus may be the primary mechanism of cross protection.

A613

THE EFFECT OF DAY LENGTH ON ALFALFA MOSAIC VIRUS MULTIPLICATION AND RNA STABILITY. S. Flasinski, O.W. Barnett, D.A. Kluepfel, Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634-0377

Alfalfa mosaic virus (AMV) strains 425, 106, and Soy were purified from *Nicotiana clevelandii* 7, 10, and 14 d after inoculation. AMV-106 virion yields were highest 7 d after inoculation while AMV-425 yields were highest at 10 d and AMV-Soy at 14 d. Yields of AMV-425 were greater from plants growing in an 18/6 photoperiod than in a 12/12 photoperiod, but not for AMV-106 and AMV-Soy. Different amounts of RNA 1, 2, 3, and 4 were purified from virions of each strain at the different harvest dates and photoperiods. In general, high virus yields resulted in intact strands of all four RNAs. Amount of RNA-4 per mg virions did not change with time of inoculation or photoperiod but RNA 1, 2, and 3 occurred in lower amounts relative to RNA-4 14 d after inoculation. Photoperiods also affected the ratios of RNA 1, 2, or 3 to RNA-4. RNA degradation, in vivo or in vitro, could explain some of these differences.

A614

PROPERTIES OF THE MENTHA STRAIN OF LYCHNIS RINGSPOT VIRUS. L. Beczner (1), R.I. Hamilton (2), and D.M. Rochon (2), (1) Plant Protection Institute, Hungarian Academy of Sciences, Budapest H-1525, Hungary, and (2) Agriculture Canada Research Station, 6660 N.W. Marine Drive, Vancouver, B.C., Canada V6T 1X2.

A hordeivirus, isolated from horsemint (Mentha longifolia Huds.) in Hungary, was designated as the mentha strain of lychnis ringspot virus (LRSV-M) on the basis of serological relationship with the type strain (LRSV-T), reciprocal nucleic acid hybridization, and similar properties of their ss (genomic) and ds RNAs. Four smaller ssRNAs in addition to the three genomic RNAs were encapsidated by LRSV-M coat protein. No hybridization was detected under high stringency conditions between randomly primed cDNA of LRSV-M and other hordeiviruses (barley stripe mosaic [BSMV] and poa semilatent [PSLV] viruses). Reciprocal hybridization experiments using cDNAs to BSMV and PSLV confirmed that the hordeiviruses are a group of morphologically similar but genetically distinct viruses.

A615

Double-stranded RNA in Alternaria solani. I. Zabalgogeazcoa, D. Petrunak, B.J. Christ, and F.E. Gildow. Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802.

High molecular weight double-stranded RNA (dsRNA) was detected in five isolates of Alternaria solani, the causal agent of early blight of potato. The five isolates were obtained from potato leaves collected in Pennsylvania, Maine, and New York. DsRNA was extracted with phenol and purified by cellulose chromatography from cultures grown in liquid media. Each isolate showed a characteristic dsRNA electrophoretic pattern in agarose gels consisting of 1 to 4 bands ranging in molecular weight from 3.2 to 18.5 x 10^6 d. Some bands were common among isolates. No virus-like particles or abnormal cytopathological structures were observed in any of two to six hyphal tips of each isolate analyzed by transmission electron microscopy.

A617

SIMULATION OF ULTRAVIOLET ABSORPTION SPECTRA OF VIRUSES AND PROTEINS. L.C. Lane, Dept. of Plant Pathology, Univ. of Nebraska, Lincoln, NE, 68583-0722.

Ultraviolet absorption spectroscopy is a convenient method of characterizing viruses and proteins. To a first approximation the UV absorption spectrum of a protein is the sum of spectra for tryptophan, tyrosine, phenylalanine and disulfide bonds. Spectra of individual proteins can be simulated by entering amino acid spectra into a computer spreadsheet and combining them in ratios dictated by the protein amino acid content.

Virus spectra can be simulated by adding, in addition, spectra for RNA and light scattering. Tryptophan/tyrosine ratios strongly influence protein spectra. Tryptophan/tyrosine ratios of unknowns can be estimated by comparing actual spectra to computer generated spectra. Comparing UV spectra of viruses to computer generated spectra is a useful criterion of purity. Generating spectra by computer is a useful way for students to learn spectroscopy principles.

A619

TRANSFORMING POTATO WITH THE COAT PROTEIN GENE OF AN RPV ISOLATE OF BARLEY YELLOW DWARF VIRUS (BYDV). C.-H. Lei, J. R. Vincent, G. A. Thompson, B. A. Larkins and R. M. Lister. Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.

We are investigating the potential for expression of the BYDV coat protein (CP) gene to protect potato against potato leaf roll virus. An expression cassette for the 22 kD-CP gene of the NY-RPV isolate of BYDV was used to transform potato (FL1607; Frito-Lay Inc.). The cassette, which included the cauliflower mosaic virus 35 S promoter, a cDNA corresponding to the RPV CP gene, and the nopaline synthase polyadenylation signal sequence, was constructed in the cloning vector pGEM-3Z and then subcloned into the binary vector pBIN19 as pRPV210. Potato leaf strips were transformed by inoculating with *Agrobacterium tumefaciens* LBA 4404 containing pRPV210, by the method of Wenzler *et al.* (Plant Science, 63:79-85). A total of 143 calli were initiated from 50 leaf strips during 30 days on callus-inducing medium with 50 mg/l kanamycin; 105 of the calli regenerated plants. None of the 25 non-inoculated control strips survived this selection. Preliminary northern analyses readily detected CP mRNA transcripts in 4 of 6 presumed transformants. Further tests for gene copy number, mRNA transcripts, and CP expression are in progress.

A620

MOLECULAR CLONING AND SEQUENCING OF THE SATELLITE VIRUS OF MAIZE WHITE LINE MOSAIC VIRUS. L. Zhang, T. A. Zitter, and P. Palukaitis, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

The satellite virus of maize white line mosaic virus (SV-MWLMV) was purified and separated from its helper virus (MWLMV) by two cycles of sucrose gradient centrifugation. The SV-RNA was extracted, poly (A)-tailed, and used as a template for cDNA cloning. Most of the SV-RNA sequence was determined from the cloned cDNA, and the remaining 50 nucleotides at the 5' end were determined by direct RNA sequencing. The SV-MWLMV genome is 1168 nucleotides long and contains a single open reading frame encoding a potential polypeptide of 23,988 Da (about the size of its coat protein, 24 kDa). A protein of the same size was produced by *in vitro* translation of the SV-RNA. This protein reacted with the antiserum against the SV-MWLMV coat protein in Western blot analysis. Computer analysis revealed no significant homology between the SV-MWLMV and any other virus or satellite RNA.

A621

A PHYTOREOVIRUS ISOLATED FROM PERIWINKLE USED AS BAIT PLANTS IN A BLUEBERRY FIELD. B.I.Hillman[1], J.V.Anzola[2], T.D.Cavileer[1], F.Fuertges[1], and D.L.Nuss[2] [1]Dept. of Plant Pathology, Rutgers University, New Brunswick, NJ 08903 and [2] Roche Institute of Molecular Biology, Nutley, NJ 07110.

In the spring of 1989, periwinkle plants (Catharanthus roseus) were set out in a New Jersey blueberry field in an effort to trap the mycoplasma-like organism associated with blueberry stunt disease. Although no MLOs were visible by electron microscopy (T.A.Chen, pers. comm.), several viruses were detected by dsRNA analysis, EM, and symptomatology. Among the viruses detected by dsRNA analysis was one that contained 12 dsRNA segments, typical of the genomes of Phytoreoviruses. Analysis by spot and northern hybridizations with cDNA clones derived from wound tumor virus, the only characterized Phytoreovirus that infects dicotyledons, indicated that this virus is related but not identical to WTV.

A622

SPECIFIC ENDORIBONUCLEASE CLEAVAGE OF TMV GENOMIC RNA BY AN ENGINEERED RIBOZYME. B.V. Edington, A.D. Choudhary, R.A. Dixon, and R. S. Nelson, The Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK 73402.

Ribozymes are RNA molecules which possess an enzymatic self-cleaving or self-splicing activity. Properties of this self-cleaving activity may be used in the design of endoribonucleases, with the potential of altering gene expression. Such a ribozyme has been constructed and targeted to cut the plus strand of TMV at nucleotide 2467, which will bisect the coding region of the TMV RNA-dependent RNA polymerase. The ribozyme itself is flanked by 40 nucleotides which are complementary to the TMV sequences surrounding the cleavage site. This RNA enzyme has been used in in vitro assays to cleave purified positive strand TMV RNA. Experiments are now in progress to assay the ability of this ribozyme to cleave TMV genomic RNA in tobacco protoplasts, and thus prevent viral replication.

A623

CONSTRUCTION OF CDNA PROBES FOR THE DETECTION OF TOMATO RING-SPOT VIRUS. J. R. Guo and T. A. Chen. Department of Plant Pathology, Rutgers University, New Brunswick, NJ 08903.

Complementary DNA of the New Jersey isolate of tomato ringspot virus was synthesized using reverse transcriptase with random primers. The double stranded DNA was cloned into pUC 18 and transformed into E. coli DH5α cells. 116 recombinant plasmids were selected by colony hybridization using the double stranded cDNA as a probe. Cloned inserts ranged from 0.3 to 2.6 kb. comparison of restriction enzyme maps and southern hybridization patterns of 8 clones indicated that they represented about 56% of the RNA genome. 5 clones were ^{32}P-labeled by nick translation and used in dot blot hybridization. The dot blot assay allowed the detection of as little as 20 pg of RNA from purified virions as well as virus in a 1:512 dilution of crude sap from infected tobacco plants. The cloned DNA probes hybridized with all 8 isolates tested. The probes will be used to detect virus infection of fruit trees and to investigate the biology of the nematodes which transmit the virus.

A624

RESISTANCE IN TRANSGENIC POTATO EXPRESSING THE POTATO LEAFROLL LUTEOVIRUS COAT PROTEIN GENE. L.M. Kawchuk (1), R.R. Martin (1), and J. McPherson (2). (1) Agriculture Canada Research Station, Vancouver, B.C. V6T 1X2 and (2) Department of Plant Science, University of British Columbia, Vancouver, B.C. V6T 1X2.

Three constructs of the potato leafroll luteovirus (PLRV) coat protein gene were inserted into the commercial potato cultivar Russet Burbank via an Agrobacterium tumefaciens mediated gene transfer. One construct possessed 12 nucleotides of the untranslated leader sequence 5' to the coat protein AUG and the other construct, which was also inserted in the reverse orientation to produce negative sense RNA, had 112 nucleotides from this leader sequence. Introduced as chimaeric genes under the control of the duplicated CaMV 35S promoter, transcription levels were high but coat protein levels were less than 0.01% of total leaf protein. Results show that significant levels of sustained resistance are obtained with each construct.

A625

MAPPING DETERMINANTS OF PATHOGENICITY AND TRANSMISSION OF CUCUMBER MOSAIC VIRUS. M.H. Shintaku and P. Palukaitis. Cornell University, Ithaca, NY 14853

Two strains of cucumber mosaic virus (Fny- and M-CMV) differ greatly with regard to aphid-transmissibility, symptom expression, and the ability to infect the squash cultivar 'Black Beauty'. With respect to these differential phenotypes, transcripts of cDNA clones of the Fny-CMV genomic RNAs 1, 2, and 3 produce infections and progeny virus typical of Fny-CMV, whereas replacing the Fny-CMV RNA 3 transcript with an M-CMV RNA 3 transcript results in infections and progeny virus typical of M-CMV. Reciprocal recombinants between the two CMV RNA 3 clones and the resulting infections on tobacco and squash implicate the coat protein gene as the determinant of these differential phenotypes. A comparison of the

nucleotide sequences of the two strains revealed 14 nucleotide differences in the coat protein genes, resulting in 8 predicted amino acid differences. Recombinants within the coat protein gene are being used to further delimit the amino acids involved in determining these phenotypes.

A626

THE GENOME OF THE NY-RPV ISOLATE OF BARLEY YELLOW DWARF VIRUS. J. R. Vincent, R. M. Lister, and B. A. Larkins. Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, 47907.

The group 2 isolate of barley yellow dwarf virus (BYDV), NY-RPV, can be distinguished from group 1 BYDV isolates by serological relationships, cytopathological ultrastructure of infected cells, and dsRNA profiles obtained from infected tissues. To investigate the genomic basis for these differences, cDNA libraries were constructed from NY-RPV viral RNA in both plasmid and bacteriophage vectors. From these libraries, overlapping clones representing the NY-RPV genome were identified by restriction analysis and by hybridization, and subsequently sequenced. The genome of NY-RPV is ~5.6 Kb in length, within which six major (+) strand open reading frames (ORFs) were identified. Based on both the sequence and the organization of the genome, NY-RPV is clearly different from group 1 BYDV isolates. Furthermore, the genome of the NY-RPV isolate of BYDV more closely resembles that of two other luteoviruses, beet western yellows virus and potato leafroll virus, than those of group 1 BYDV isolates.

A627

CHARACTERIZATION OF THE BARLEY YELLOW DWARF VIRUS NY-MAV-PS1 AND P-PAV GENOMES. P. P. Ueng, J. R. Vincent, E. Kawata, H. C. Lei, R. M. Lister, and B. A. Larkins. Dept. of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, 47907.

The NY-MAV-PS1 and P-PAV isolates of barley yellow dwarf virus (BYDV) are serologically related, but are not identical. Both BYDV isolates are transmitted by the aphid Sitobion avenae, but P-PAV is also transmitted by Rhopalosiphum padi. To evaluate the genomic basis for these, and other differences, cDNA libraries were constructed from the RNA of each BYDV isolate in both plasmid and bacteriophage vectors. From these libraries, overlapping clones representing the genome of each viral isolate were identified by restriction analysis and by hybridization, and subsequently sequenced. Each genome is ~5.2 Kb in length with six identified (+) strand open reading frames (ORFs). The greatest diversity between the NY-MAV-PS1 and P-PAV sequences was found in ORFs located at the 3' end of the respective genomes, indicating that this region of the genome may be involved in the properties which differentiate BYDV-NY-MAV-PS1 and BYDV-P-PAV.

A629

Red clover necrotic mosaic virus infectious transcripts synthesized *in vitro* from full-length cDNA clones. Z. Xiong, and S. A. Lommel, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC, 27695-7616.

The red clover necrotic mosaic virus (RCNMV) genome is split among two non-homologous ssRNAs of 3.9 kb (RNA-1) and 1.5 kb (RNA-2). Near to full length cDNA clones were generated to both RNAs. The clones were determined to be short of full length by several nucleotides at both termini. Oligo-directed mutagenesis was employed to incorporate the missing 5' terminal nucleotides as well as fuse the pBS(+) T7 promoter to both clones. Authentic viral RNA-1 and -2 begin with a m^7GpppA. The T7 promoter extends one nucleotide into the transcript sequence, preferring that the transcript begins with a guanosine. Consequently, an additional non-viral guanosine residue was engineered to the 5' end of the viral sequence. The same approach was taken to incorporate the non-representative nucleotides at the 3' terminus of the clones as well as incorporate a convenient *SmaI* restriction site. Following *SmaI* restriction and T7 transcription in the presence of m^7GpppG, infectious RNA-1 and -2 run-off transcripts were made. Inoculation of the RNA-1 and RNA-2 *in vitro* transcripts resulted in systemic infection and typical symptom formation on *Nicotiana clevelandii*.

A630

Wheat Streak Mosaic Virus Genomic RNA Shares Sequence Homology with Potyviral Cylindrical Inclusion Cistrons. K. R. Zagula, T. L. Kendall and S. A. Lommel, Department of Plant Pathology, North Carolina State University, Raleigh, N.C. 27695-7616.

Wheat streak mosaic virus (WSMV), a putative member of the potyvirus group, has a single-stranded RNA genome of approximately 8.5 Kb. To date, the capsid protein cistron has been mapped proximal to the 3' terminus. A 1.0 Kb cDNA clone not 3' co-terminal with clones containing capsid protein coding sequence was synthesized by oligo-dT priming. The nucleic acid sequence of this clone has been determined, and its amino acid sequence deduced. The amino acid sequence shares homology with tobacco etch, plum pox, and tobacco vein mottling viruses and potato virus Y within the N terminus of the cylindrical inclusion protein. The consensus sequence for nucleotide binding, GXXGXGKS, which is highly conserved among the four sequenced potyviruses, is present at the 5' terminus of the WSMV clone. These data further support the inclusion of WSMV within the potyvirus group.

A631

MOLECULAR ANALYSIS OF TOBACCO VEIN MOTTLING VIRUS (TVMV) PATHOGENICITY BY INFECTIOUS TRANSCRIPTS OF CHIMERIC POTYVIRAL cDNA GENOMES. Gary M. Hellmann[1], David W. Thornbury[2], and Thomas P. Pirone[2]. Bowman Gray Technical Center, RJR Nabisco Inc., Winston-Salem, NC 27102[1], and Plant Pathology Department, University of Kentucky, Lexington, KY 40546[2].

The tobacco cultivar TN86 is "resistant" to most isolates of TVMV, the virus being restricted to epidermal cells of inoculated leaves. A variant, TVMV-S, overcomes the resistance and infects TN86 systemically. To investigate the molecular basis for the ability of TVMV-S to move systemically in TN86, viral RNAs obtained from TVMV and TVMV-S were isolated and translated *in vitro*. SDS-PAGE analysis of the translation products revealed strain differences in the apparent MWs of two non-structural proteins- 34K and NIb. cDNA clones were prepared from TVMV-S RNA and used to construct a chimeric potyvirus cDNA genome possessing the 5'-terminal (34K-HC-42K-CI) cistrons of TVMV and the 3'-terminal (6K-NIa-NIb-CP) cistrons of TVMV-S. Chimeric and wild-type transcripts readily infected a cultivar (KY14) susceptible to both TVMV and TVMV-S, but both failed to infect TN86. These results suggest that gene(s) responsible for resistance-breaking in TN86 are encoded by 5'-terminal sequences of TVMV-S. This experimental system holds the potential for identifying a potyviral "movement protein" as well as identifying host polypeptides with which it may interact.

A632

HETEROLOGOUS ENCAPSIDATION IN MIXED INFECTIONS AMONG THREE ISOLATES OF BARLEY YELLOW DWARF VIRUS. F. Wen and R. M. Lister. Dept. Botany and Plant Pathology, Purdue Univ., W. Lafayette, IN 47907.

Immunohybridization (J. gen. Virol. 71:211) and ELISA were used to study heterologous encapsidation between paired isolates of barley yellow dwarf virus (BYDV) in mixedly infected oat plants. One-way heterologous encapsidation was detected between NY-RPV and NY-MAV-PS1 isolates, and between NY-RPV and P-PAV isolates. Apart from homologous encapsidation, some of the RNAs of either P-PAV or NY-MAV-PS1 were heterologously encapsidated in the protein capsids of NY-RPV to form "hybrid" virions, but there was no evidence of such encapsidation of the RNAs of NY-RPV in the protein capsids of either P-PAV or NY-MAV-PS1. Two-way heterologous encapsidation was detected between P-PAV and NY-MAV-PS1, i.e. some of the viral RNAs of P-PAV or NY-MAV-PS1 were detected in virions trapped with NY-MAV-PS1-specific or P-PAV-specific antibodies, respectively. Further analysis, including two-site ELISA, to determine whether the heterologous encapsidation involves transcapsidation, phenotypic mixing, or both, is in progress.

A634

LACK OF SEQUENCE HOMOLOGY BETWEEN THE GENOMIC RNA OF THE BACILLIFORM VIRUS IN *AGARICUS BISPORUS* AND THE LA FRANCE DISEASE-RELATED DOUBLE-STRANDED RNAS. C. P. Romaine, B. Schlagnhaufer, and M. Goodin, Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802.

La France disease of *Agaricus bisporus* is associated with a conserved electrophoretic pattern of nine dsRNAs. In addition, several virus-like particles, including a 19 x 50 nm ssRNA bacilliform virus (MBV), have been detected in diseased tissues. We investigated the relationship between MBV RNA and the dsRNAs. Upon electrophoresis in denaturing 1.5% agarose gels, the genome of MBV migrated as a single 4.4 kb RNA molecule. A 1.4 kb cDNA to MBV RNA was synthesized by oligo (dT)-primed reverse transcription, cloned, and radiolabeled by random priming. Northern blot analysis showed that the cDNA probe hybridized to the genomic RNA isolated from purified virus, but not to a comparable fraction from healthy basidiocarps. Similarly, the probe detected MBV RNA sequences in dot blots of total nucleic acid fractions from diseased, but not healthy, basidiocarps. No sequence homology existed between the cDNA and either the dsRNAs associated with La France disease or those present in healthy tissues. The results suggest that MBV is distinct from the putative dsRNA viruses.

A635

MOLECULAR CLONING OF COWPEA MOTTLE VIRUS CAPSID GENE. J. W. Kim and R. F. Bozarth. Dept. of Life Sciences, Indiana State Univ., IN 47809.

Cowpea mottle virus (CMeV) is a non-enveloped plant virus reported only from Nigeria. It has one (+)-ssRNA as a genome (mol. wt. 1.4 x 10^6 [4 Kb]) and one capsid protein (mol. wt. 40 K). cDNAs were generated using random primers and cloned into phagemid vector pT7/T3 18U. The insertion ranged from 500 bp to 2100 bp. A CMeV capsid gene sequence was obtained from clones pCMeV-16S and pCMeV-12. The CMeV capsid gene is composed of 1104 nt and the codons represent 367 amino acids. The amino acid sequence deduced from the nucleotide sequence of capsid gene was compared to those of carmoviruses. The S-domain showed about 30% sequence homology eventhough Western blot and Northern blot analysis showed no cross-reaction among CMeV and carmoviruses.

A636

CONSTRUCTION AND ANALYSIS OF A VIRAL VECTOR FOR RAPID ANALYSIS OF GENE EXPRESSION IN WHOLE PLANTS. B. Cassidy and R. Nelson, The Noble Foundation, P.O. Box 2180, Ardmore, OK 73402.

Elements presumed to be necessary for replication and translation from an attenuated strain of tobacco mosaic virus (TMV) were isolated from cDNA clones or chemically synthesized and combined to create an expression vector. This vector has characteristics of both defective interfering particles and satellite viruses. It contains DNA homologous to the TMV origin of assembly sequence and the 3' untranslated region. A series of restriction sites have been incorporated to allow the insertion of foreign genes. When coinfected with an attenuated strain of TMV these elements will allow for replication and encapsidation of the vector RNA and foreign gene expression. Expression of foreign genes will allow the observation of effects in differentiated tissues and at the same time avoid the long delays in analysis when producing transgenic plants. The expression and systemic movement of a reporter gene, β-glucuronidase, will be presented.

A637

UPSTREAM SEQUENCES IN ADDITION TO AAUAAA ARE REQUIRED FOR EFFICIENT FUNCTIONING OF TWO PLANT POLYADENYLATION SIGNALS. B. D. Mogen, M.H. MacDonald, R. Graybosch, and A.G. Hunt. Plant Physiology/Biochemistry/Molecular Biology Program, Department of Agronomy, University of Kentucky, Lexington, KY 40546-0091.

We have characterized the upstream sequences needed for functioning of the polyadenylation signals from cauliflower mosaic (CaMV) virus and a pea ribulose-1,5-bisphosphate carboxylase small subunit gene. Sequences between 53 and 181 bases upstream from the CaMV polyadenylation site are required for efficient polyadenylation at this site, as is an AAUAAA sequence located 16-21 bases upstream from this site. An element located between 60 and 137 bases upstream from the poly(A) addition sites in a pea ribulose-1,5-bisphosphate carboxylase small subunit (*rbc*S) gene is needed for functioning of these sites, indicating that upstream sequence elements far from polyadenylation sites may be a common feature in plant genes. Our studies indicate that upstream sequences other than, or in addition to, AAUAAA are essential for mRNA 3' end formation in these two genes. We suggest that multiple elements are involved in mRNA 3' end formation in plants. These components of the plant polyadenylation apparatus appear to interact with their respective sequence elements and with each other to result in efficient mRNA 3' end formation.

A638

RELATEDNESS BETWEEN AN OPINE CATABOLIC GENE AND A T-DNA OPINE BIOSYNTHETIC GENE. S.B. Hong[1], Y. Dessaux[2], P. Guyon[2], J. Tempé[2] and S.K. Farrand[1]. [1]Dept. Plant Pathol. Univ. Illinois, Urbana, IL. 61801 and [2]Instit. Microbiol. Univ. Paris-Sud, Orsay 91405, France.

Crown gall tumors induced by octopine-type *Agrobacterium tumefaciens* strains produce four mannose-containing opines. One of these, called agropine (AGR), is a lactone of a second, called mannopine (MOP). We now show that extracts from tumors producing agropine contain an activity that cyclizes MOP to AGR and that this activity is the product of T_R gene 26. Strains harboring octopine-type Ti plasmids also contain a catabolic activity that lactonizes MOP to AGR. This cyclase is encoded by a Ti plasmid gene unlinked to the T-DNA. A fragment encoding the catabolic MOP cyclase was subcloned from the Ti plasmid and the gene delimited by deletions. The region was sequenced and an open reading frame encoding a predicted 45.5 kd protein was identified. In a coupled transcription-translation system the subclone yielded a single protein with a molecular size estimated at 45 kd. The derived amino acid sequence of the proteins from T_R gene 26 and the catabolic cyclase gene show 53% identity and 79% homology including conserved changes.

A639

DEVELOPMENT OF A DNA PROBE TO DETECT COPPER-RESISTANCE GENES IN XANTHOMONAS CAMPESTRIS PV. VESICATORIA. S. Garde and C. Bender, Dept. of Plant Pathology, Oklahoma St. Univ., Stillwater, OK 74078.

We recently demonstrated that Cu^r gene(s) in X. campestris pv. vesicatoria XV10 are encoded by a large (190 kb) conjugative plasmid designated pXV10A (Appl. & Environ. Microbiol. 56:170-175). A cosmid library of pXV10A DNA was constructed in pLAFR3. A 44 kb cosmid clone, designated pCuR1, conferred Cu^r to a copper-sensitive strain of Xcv when it was transformed by electroporation. Subcloning and transposon mutagenesis experiments were performed to further characterize the specific location and size of the Cu^r determinant on pCuR1. Several probes constructed from DNA fragments internal to the Cu^r region were hybridized to Cu^r and Cu^s Xcv strains as well as other pathogenic and saprophytic bacteria to assess their specificity for Cu^r in Xcv.

A640

GENETIC EVIDENCE FOR A TRANS-ACTING FACTOR OF ERWINIA CAROTOVORA SUBSP. CAROTOVORA (ECC) THAT STIMULATES THE PRODUCTION OF EXTRACELLULAR DEGRADATIVE ENZYMES. H. Murata and A. K. Chatterjee, Department of Plant Pathology, University of Missouri, Columbia, Missouri 65211.

Ecc strain 71 produces extracellular enzymes such as pectate lyase (Pel), polygalacturonase (Peh), cellulase (Cel), and protease (Prt). Using Tn*5*, Tn*phoA*, and Tn*10*-*lacZ*, we isolated pleiotropic mutants deficient in these enzymatic activities. The phenotype did not result from insertions in genes specifying enzyme export (*out*), adenyl cyclase, or *c*AMP receptor protein. We isolated a cosmid, pAKC264 that restored extracellular enzyme production in all mutants. In addition to complementing Tn-mutations, pAKC264 or its subclone, pAKC602, stimulated production of Pel, Peh, Cel and Prt in Ecc71 by ca. 3-fold. pAKC602 also stimulated the production of Pel, Peh and Cel in *E. coli* strains carrying the cognate structural genes. The gene was designated as *aep* for the activation of extracellular protein production.

A641

POLYGALACTURONASE PRODUCTION BY *AGROBACTERIUM TUMEFACIENS* BIOVAR 3. P. Rodriguez-Palenzuela[1], R. G. McGuire[1], A. Collmer[1] and T. J. Burr[2]. [1]Dept. of Plant Pathology, Cornell Univ., Ithaca, NY 14853. [2]Dept. of Plant Pathology, New York State Agricultural Experiment Station, Cornell Univ., Geneva 14456.

A. tumefaciens biovar 3 causes both crown gall and root necrosis of grape. Activity-stained isoelectric focusing gels of culture supernatants show the production of a single polygalacturonase (PG) by biovar 3 strains from different geographical origins but reveal no pectolytic activity from biovars 1 and 2. The pH optimum and pI of the PG are around 4.5. The enzyme is produced in Kado 523 medium, but not minimal medium, is largely extracellular, and is produced independently of the Ti plasmid. A PG with a pI of 4.5 was recovered from lesions in grape seedlings infected with *A. tumefaciens* biovar 3 strain CG49, but not from healthy seedlings. Several PG-deficient Tn*5* mutants of strain CG49 have been isolated and will facilitate further characterization of the role of the PG in *Agrobacterium* pathogenesis.

A642

BROAD-HOST-RANGE COSMID AND PLASMID VECTORS FOR USE IN *PSEUDOMONAS*. D. W. Bauer and A. Collmer. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

A new cosmid vector, pCPP34, was constructed from the broad-host-range, IncP vector, pMP92 (Spaink et al. 1987. Plant Mol. Biol. 9:27-39). The cosmid is small (ca. 8.9 kb) and contains two *cos* sites flanking a unique blunt-end restriction site, thus simplifying construction of libraries. When packaged, 1.5 kb of the vector is lost, allowing inserts of 30-45 kb to be cloned. The vector contains a *Bam*HI cloning site flanked by T3 and T7 promoters and *Not*I sites for rapid mapping of inserts. The vector was mobilized from *Escherichia coli* into *Pseudomonas syringae* pv. *syringae* where it was similar in stability to pLAFR5. The cosmid has been used to construct libraries of *P. syringae* pv. *syringae* and *P. syringae* pv. *lachrymans*. Several additional derivatives of pMP92 were constructed which contain the lacα region from pUC19 or pUC128 and have been useful in subcloning fragments of *P. syringae* DNA due to their small size (ca. 7.2 kb) and ability to stably maintain inserts.

A643

SITE-DIRECTED MUTAGENESIS OF CORONATINE SYNTHESIS GENES IN PSEUDOMONAS SYRINGAE PATHOVARS GLYCINEA, ATROPURPUREA, AND MORSPRUNORUM. C. L. Bender and S. A. Young, Dept. of Plant Pathology, Oklahoma St. Univ., Stillwater, OK 74078.

In P. syringae pv. tomato PT23.2, plasmid pPT23A (101 kb) is involved in synthesis of the phytotoxin coronatine. The characterization of Tn5 insertions and deletions in pPT23A suggest that a 30 kb region of this plasmid is necessary for coronatine production. Coronatine synthesis genes in pathovars atropurpurea, glycinea and morsprunorum were mutated using Tn5-inactivated sequences from the 30 kb region of pPT23A. Physical characterization of the mutations which resulted indicated that genes controlling coronatine synthesis in pv. atropurpurea 1304, glycinea 4180, and morsprunorum 567 and 3714 are located on large (90-105 kb) indigenous plasmids. Therefore, coronatine biosynthesis genes are strongly conserved in the plasmid DNAs of four producing pathovars, despite their disparate origins (California, Japan, New Zealand, Great Britain and Italy).

A644

VOLATILE COMPOUNDS INDUCE EXTRACELLULAR POLYSACCHARIDE PRODUCTION BY A *PSEUDOMONAS SOLANACEARUM* MUTANT. S. C. Clough and T. P. Denny, Dept. of Plant Pathology, University of Georgia, Athens, GA 30602.

Mutant AW1-83, derived from *Pseudomonas solanacearum* strain AW1 by insertion of an IS*50* element, has a pleiotropic phenotype similar to that of a spontaneous phenotype conversion mutant. Extracellular polysaccharide (EPS) production by AW1-83 is drastically reduced when cultured alone, but is reversibly induced to normal levels when AW1-83 is grown on a split-plate adjacent to the wild-type strain AW1. The inducer produced by AW1 is volatile, but is not ethylene. A volatile compound from tomato fruit as well as a relatively high amount of methanol vapor also induce EPS production by AW1-83. Work to identify the naturally-produced volatile inducer from strain AW1 and isolate the *P. solanacearum* wild-type gene responsible for the inducible phenotype of AW1-83 are in progress.

A645

CHARACTERIZATION OF PLANT SIGNALS THAT INDUCE THE *SYRB* GENE REQUIRED FOR SYRINGOMYCIN PRODUCTION BY *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE*. Y.-Y. Mo, R. F. Bonsall, and D. C. Gross, Dept. Plant Pathology, Washington State Univ., Pullman 99164.

A *syrB::lacZ* gene fusion, obtained by Tn*3*HoHo1 mutagenesis, was marker exchanged into strain B3A-R of *Pseudomonas syringae* pv. *syringae*. The resultant strain, B3AR132, expressed high β-galactosidase activity only when specific plant signal molecules were present in the culture medium. Signals that transcriptionally activated *syrB::lacZ* were extracted from cherry leaves and purified by C-18 reverse-phase HPLC. Most of the signal activity was associated with phenolic compounds exhibiting absorbancy maxima near 206, 266 and 348 nm. However, a nonphenolic peak enhanced the activity of the purified phenolic signals from cherry leaves by over 10 fold. The enhancer fraction also caused a 4- to 10-fold increase in the signal activities of arbutin, esculin and salicin, previously shown to induce the *syrB::lacZ* fusion. The chemical and biological properties of the cherry signals will be discussed.

A646

MOLECULAR CLONING AND CHARACTERIZATION OF A PECTINASE GENE FROM A PLASMID-CARRYING STRAIN OF PSEUDOMONAS VIRIDIFLAVA. K. Sasaki, C.-H. Liao, G. Nagahashi and K.B. Hicks, ERRC, USDA-ARS, Philadelphia, PA 19118

A pectinase gene from a plasmid-carrying strain (SJ074) of Pseudomonas viridiflava was cloned and expressed in E. coli using pBR322 as a vector. All four resulted pectolytic transformants produced only one type of pectate lyase with pI 9.7 and no endopolygalacturonase. The smallest insert (9-10 kb) from one of the four clones was restricted with various endonucleases and a restriction map of the cloned fragment was constructed. One of the SphI subfragment (3.6-3.7 kb) encoded the pectate lyase gene (pel). Further investigations are being made to determine if the pel is plasmid-born and if pel homologs exist in other strains of P. viridiflava.

A647

MOLECULAR CHARACTERIZATION OF A MUTANT FROM PSEUDOMONAS VIRIDIFLAVA THAT CAUSES HYPERSENSITIVE-TYPE NECROSIS ON PEPPER FRUITS. C.-H. Liao and K. Sasaki, ERRC, USDA-ARS, Philadelphia, PA 19118.

P. viridiflava is a postharvest pathogen, which causes soft rot of vegetables by producing a single pectate lyase (PL; pI 9.7). We have previously shown that insertion of transposons into genes controlling synthesis and secretion of PL results in the loss of pathogenicity. Here we describe another pathogenicity-related mutant, which produces PL normally in cultures but fails to induce soft-rot symptoms on plants. When inoculated onto pepper fruit, this mutant causes browning and necrosis of tissues. The mutation is pleiotropic and reversible at relatively high rates. A genomic library of the parent strain has been constructed in pLAFR3 and will be used to identify genes resoponsible for induction of this hypersensitive-type response in host plants.

A648

CHARACTERIZATION AND ISOLATION OF GENES CODING FOR TOXIN PRODUCTION IN *BACILLUS THURINGIENSIS*. M. C. Kosinski, D. A. Kluepfel, and G. R. Carner, Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634-0377.

B. thuringiensis is a gram-positive bacterium which produces a protoxin crystal during sporulation. When ingested by lepidopteran larvae, the crystal becomes an endotoxin deadly to lepidopterans but not to other organisms. Bioassays to determine the LD 50 of four strains of *Bacillus thuringiensis* (HD-1, HD-73, HD-187 and HD-263) against *Heliothis virescens* showed that strain HD-263 was two to seven times more effective against *H. virescens* than the benchmark strain HD-1. Chromosomal and plasmid DNA from HD-263 were extracted and purified; DNAs were separated on 0.7% agarose gels, transferred to nitrocellulose paper by the Southern blot procedure, and probed with a ^{32}P-labelled, truncated *B. thuringiensis* clone from strain *B. thuringiensis* subspecies *berliner* 1715. The delta endotoxin gene in strain HD-263 was identified, located, and cloned to allow study of its elevated level of insecticidal activity.

A649

A PATHOGENICITY GENE FROM *ERWINIA AMYLOVORA* ENCODES A PREDICTED PROTEIN PRODUCT HOMOLOGOUS TO A FAMILY OF PROCARYOTIC RESPONSE REGULATORS. B. J. Sneath, J. M. Howson, and S. V. Beer. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

A cluster of *hrp* genes from *Erwinia amylovora* spaning ca. 40 kb was cloned previously. The naturally occurring Hrp$^-$ strain P66 (from E. Billing) has been complemented by a 2.7 kb BamHI-HindIII fragment from the middle of the *hrp* cluster. The nucleotide sequence of the fragment was determined. The predicted protein product from this fragment is homologous to members of the superfamily of procaryotic response regulator proteins. The proteins HrpS from *Pseudomonas syringae* pv. *phaseolicola*, TyrR from *Escherichia coli*, NtrC from *Bradyrhizobium* sp. and *Rhizobium meliloti*, and NifA from *Klebsiella pneumoniae* and two *Rhizobium* species, are significantly similar to the predicted *E. amylovora* Hrp protein. Studies of the role of this *hrp* locus in the regulation of other *hrp* genes in the cluster are in progress.

A650

THE *HRP* GENE CLUSTER OF *ERWINIA AMYLOVORA* SHARES DNA HOMOLOGY WITH OTHER BACTERIA. R. J. Laby and S. V. Beer. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

The entire *hrp* gene cluster of *E. amylovora* was cloned previously as a 46 kb insert in cosmid pCPP430. Subclones from the cosmid were used as probes of Southern blots. Following low stringency (47°-57°C) washes, hybridization was observed between certain subclones and genomic DNA of *E. carotovora*, *E. chrysanthemi*, *E. lupinicola*, *E. mallotivora*, *E. nigrifluens*, *E. rubrifaciens*, *E. salicis*, and *E. stewartii*. Hybridization between certain subclones also occurred with pES1044, a cosmid containing genes of *E. stewartii* involved in water-soaking. With higher stringency (65°C) washes, hybridization was observed only between subclones and *E. amylovora*. Certain subclones hybridized with pHIR11, a cosmid containing the *hrp* cluster of *Pseudomonas syringae*, and with genomic DNA of *P. syringae* at low stringency. These results indicate that the *hrp* gene cluster of *E. amylovora* shares DNA homology with other phytopathogenic bacteria, including some that do not elicit the HR.

A651

BIOLISTIC TRANSFORMATION OF A PROKARYOTE, *BACILLUS MEGATERIUM*.
Franzine D. Smith, Katherine B. Shark, Peter R. Harpending, and John C. Sanford
Dept. of Horticultural Science, Cornell Univ., Geneva, NY 14456.

We have developed biolistic methods for transforming *B. megaterium* strain 7A17 with plasmid pUB110. This is the first report of biolistic transformation of a prokaryote. Cells were spread on solid LB medium plus 50 µg/ml methionine and sorbitol (0 -1.75 M), bombarded with DNA-coated (0.8 µg DNA per bombardment) tungsten particles (0.1 µm-1.0) and then overlayed with LB containing 50 µg/ml kanamycin. Transformation efficiency was highest when the cell density was 7.5×10^7 cfu/9 cm plate and sorbitol concentration was 1.25 M. The number of transformants at 1.25 M sorbitol was 5.5 times greater than at 0.75 M. Transformation was confirmed by restriction and gel electrophoresis of plasmid DNA. An improved particle accelerator design dramatically increased transformation efficiency. Hundreds (>500) of transformants per petri dish were produced as compared to <10 with the old particle accelerator. The effects of bacterial strain, pre- and post-bombardment treatment of cells, and DNA size are being tested as well as transformation of other species. Particle bombardment may prove to be a universal process for transformation of prokaryotes as well as eukaryotes.

A652

FUNCTIONAL HOMOLOGY BETWEEN A LOCUS OF *ESCHERICHIA COLI* AND THE *HRP* GENE CLUSTER OF *ERWINIA AMYLOVORA*. Z.-M. Wei and S. V. Beer. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

A cosmid (pCPP430) containing the entire *hrp* gene cluster of *E. amylovora* was identified previously. Strains of *E. coli* harboring pCPP430 elicit the hypersensitive response (HR) in tobacco and other plants. Two other cosmids pCPP440 and pCPP450, which do not complement Hrp^- mutants at the left end of the cluster, also bestow on strains of *E. coli* the ability to elicit the HR. A 12.5 kb EcoRI fragment from the left end of pCPP430 was mutagenized with a transposon and the insertions marker-exchanged into the chromosome of *E. coli*. Mutant *E. coli* transconjugants containing pCPP440 or pCPP450 failed to elicit the HR. However, the HR was restored (in trans) by a 2.9 kb HindIII subclone from the left end of pCPP430. These results indicate that some genes of *E. coli* can functionally complement the left-hand region of the *hrp* cluster of *E. amylovora*.

A653

APPARENT RESISTANCE TO SCLEROTINIA STEM ROT IN OILSEED BRASSICA. D.V. Phillips[1], P. L. Raymer[1], and D. L. Auld[2]
[1]University of Georgia, Georgia Experiment Station, Griffin, GA. and [2]University of Idaho, Moscow, Idaho.

Oilseed Brassicas from the USDA World collection were planted in a field naturally infested with Sclerotinia sclerotiorum at Calhoun, GA. Plants began dying in late March and over 57% of the plants were killed by Sclerotinia stem rot. Five of 380 lines tested had all plants killed and 5 lines had no plants with symptoms. There was a correlation (r=-0.53, P=.0001) between date of initial flowering and percent mortality. Late flowering lines may have escaped infection because petal fall, ascospore discharge, and high moisture conditions did not coincide. Lines flowering in early March were the most heavily damaged, with 50% of the lines with mortalities above 75% and 85% of the lines with mortalities of 50% or higher. Several lines which flowered during that period had fewer than 25% plants damaged by stem rot and are being evaluated as sources of genes for resistance to Sclerotinia stem rot.

A654

DENSITY OF SCLEROTIA OF RHIZOCTONIA SOLANI AND INCIDENCE OF SHEATH BLIGHT IN MISSISSIPPI RICE FIELDS. J.P. Damicone, M.V. Patel, and W.J. Moore. Mississippi State University, Stoneville, MS 38776

Sixty-seven fields representing various rotation sequences of rice with soybean were sampled over 2 yr for pre-plant levels of sclerotia of R. solani and incidence of sheath blight in 'Lemont' rice. Sclerotial densities were positively correlated with percent diseased tillers (PDT) (r=0.54-0.63) and percent disease foci (PDF) (r=0.64-0.67). Adjustment of sclerotial density for viability did not increase the degree of correlation with PDT or PDF. In the 3-yr period prior to sampling, years cropped to rice was generally correlated with increased sclerotial density and viability and incidence of sheath blight. Relationships for years cropped to soybean were converse to those of rice. Determination of preplant sclerotial density was not a sufficiently accurate method for predicting development of sheath blight. Rotation with soybean in an area where occurrence of soybean aerial blight is rare also did not appear to increase inoculum of R. solani or incidence of sheath blight in rice.

A655

EFFECT OF MULTIPLE DISEASE INFESTATION ON THE AGRONOMIC PERFORMANCE OF A SOYBEAN CULTIVAR. R. P. Pacumbaba and W. Tadesse. Department of Plant and Soil Science, Alabama A&M University, Normal, AL 35762.

Disease infestation by cyst nematode (SCN), stem canker (SSC), bacterial blight (BBS), combination of these diseases, and their effect on the agronomic performance of soybean cv Bragg were studied in the field from 1985-1988. The mean of three-year study for the various treatments: disease infestation of each of SCN, SSC, BBS, combination infestations of SSC+SCN, SSC+BBS, SCN+BBS, and SSC+SCN+BBS showed no significant differences on the number of nodules and protein content of the seeds when compared with the control. Significant negative correlations was observed between disease rating-protein content of the leaves, and highly significant negative correlations were observed between size of nodules-disease rating and infestation, number of pods-plant height and protein content of the leaves, plant height-disease infestation, disease rating-protein content of the leaves, and disease infestation-protein content of the leaves. Significant positive correlations were observed between the size of nodules-protein content of the leaves, number of pods-yield, and protein content of the leaves-yield. Highly significant correlations were observed between number of pods-disease infestation, plant height-protein content of the leaves, disease rating-disease infestation, and protein content of the leaves-yield. No synertistic effects were observed on soybean cv Bragg affected with combination of diseases.

A656

TIME-TEMPERATURE RELATIONSHIPS IN THE DEVELOPMENT OF SEEDLING DISEASE IN WATER-SEEDED RICE. R. A. Thompson, R. W. Schneider, and S. S. Quisenberry. Departments of Plant Pathology & Crop Physiology and Entomology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

Seedling disease, caused by Pythium spp., is a major cause of stand failure in water-seeded rice. The disorder has been associated with cool weather shortly after planting. Hourly soil temps and emergence data collected from sequential plantings in field soil, either untreated or treated with metalaxyl, were used to construct correlation matrices in order to assess the relationships between relative percent emergence (RPE) and mean, min, and max temps, number of hrs within 2.8 C temp ranges, and number of hrs below certain temps for up to 8 days after planting. There were significant inverse linear relationships (r) between RPE and number of hrs below 21.1 (-0.886), 23.9 (-0.925), and 26.7 C (-0.886) during the first 4 days after planting. Cumulative hrs within 2.8 C temp ranges were not significantly correlated with RPE.

A657

EFFECT OF TEMPERATURE ON THE RATE OF INFECTION OF SOYBEAN SEEDLINGS BY PHOMOPSIS LONGICOLLA. J.C. Rupe, University of Arkansas, Fayetteville, 72701.

The infection rate of 2-wk-old soybean seedlings (cv Forrest) by Phomopsis longicolla was determined at temperatures ranging from 15 to 35 C. The percentage of plant segments infected with P. longicolla was determined on potato dextrose agar, and rates of infection were determined by linear regression for each temperature. All regression equations were significant (P<0.0001), and coefficients of determination (r^2) varied from 0.76 to 0.96. The optimum temperature for infection was 30 C followed by 25, 20, 35, and 15 C, in that order. The results were incorporated into a previously published model relating field infection of soybean seedlings to environmental conditions. The infection rate data improved the fit of the model from a coefficient of determination (r^2) of 0.73 to 0.79.

A658

THE DEVELOPMENT OF SUBCUTICULAR LATENT INFECTION STRUCTURES BY *PHOMOPSIS LEPTOSTROMIFORMIS* ON LUPINS. P.M.Williamson, K.Sivasithamparam, and W.A.Cowling. University of Western Australia, Nedlands, W.A. 6009

Phomopsis leptostromiformis (teleomorph *Diaporthe woodii*) forms latent infections on stems of narrow-leafed lupins (*Lupinus angustifolius*) that normally develop into lesions on senescing plants. The fungus produces a hepatotoxin during colonization of stems that causes lupinosis in grazing animals.

Only 87% of viable conidia germinated on the stem epidermis, and germ tube length was short (5-30um). The infection process was found to be arrested upon penetration of the cuticle and the formation of subcuticular coralloid hyphae. Seven days after inoculation of stems of susceptible cv. Yandee the coralloid structures ranged in diameter from 25 to 50 um. Penetration was only observed immediately beneath the conidia and never below germ tubes. No appressoria were visible. Symptoms developed on the senescing host only after colonization of the stem tissue from the coralloid hyphae. These latent coralloid structures have not been reported previously for *Phomopsis leptostromiformis*.

A659

EFFECT OF SOIL TEMPERATURE ON INFECTION OF SOYBEAN ROOTS BY SCLEROTIA-FORMING ISOLATES OF COLLETOTRICHUM TRUNCATUM, Mahmood Khan and J. B. Sinclair, Dept. of Plant Pathology, University of Illinois at Urbana-Champaign, Urbana, IL 61801-4709.

Root infection of three soybean cvs. by sclerotia-forming isolates of Colletotrichum truncatum (Ct), cause of foliar anthracnose on soybeans, at four soil temperatures was studied. Plants of cvs. Boon, resistant; A. K. Kansas (AKK), moderately resistant; and Williams 82, susceptible to foliar anthracnose, were grown in the greenhouse (28± 6C) in soil infested with Ct (80 sclerotia/g soil) in water temperature tanks at 20, 25, 30, and 35C. One month after planting, disease incidence for Boon ranged from 33-50%; for AKK 33-66%, and for Williams 82 25-83%; and disease severity (scale 1-5) for Boon ranged from 0.25-1.84%,for AKK 0.25-1.00%, and for Williams 82 0.57-2.27%. Generally, roots of Boon were resistant, those of Williams 82 susceptible, and of KK intermediate. However, the reaction of AKK was variable at 30 and 35C.

A660

COLONIZATION OF SOYBEAN PODS AND SEEDS BY CERCOSPORA KIKUCHII AT DIFFERENT REPRODUCTIVE STAGES, F. A. Fernandez and J. B. Sinclair, Dept. of Plant Pathology, University of Illinois, 1102 S. Goodwin Avenue, Urbana, IL 61801-4709.

In two separate field experiments, individual plants of soybean cvs. Corsoy 79 and Williams 82 were spray-inoculated with a conidial suspension ($3.2x10^5$) of an isolate of Cercospora kikuchii (Ck) at growth stages R2, R3, R4 and R6. Pods and seeds from inoculated and uninoculated plants were harvested at maturity and bioassayed separately. On pods, the earlier the inoculation, the larger the area of pod colonization in both cvs., but the extent of colonization was greater on Williams 82 than on Corsoy 79. At later inoculations, there was an increase in the occurrence of Phomopsis spp. with a concommitant decrease in Ck on pods but not on seeds. The incidence of purple seed stain was negligible in both cvs., but Ck was recovered from asymptomatic seeds. There was no relationship between pod infection and percentage recovery of Ck from seeds.

A661

CHARACTERIZATION OF RESISTANCE IN SUNFLOWER TO MACROPHOMINA PHASEOLINA, THE CAUSE OF CHARCOAL ROT. I. Ahmad, K. Burney, and P. S. Dyer*. Crop Diseases Research Institute, PARC, P.O.Box 1031, Islamabad, Pakistan and *The Botany School, Downing Street, Cambridge, CB2 3EA, U.K.

Four sunflower hybrids, NK-212, Cargill-204, SF-100 and Hysun-30 were tested for disease resistance against 26 isolates of Macrophomina phaseolina collected from sunflower growing areas in Pakistan. Stem inoculations were made using a tooth-pick method and spread of charcoal rot was measured from the point of inoculation. Disease reaction was scored on a 0 to 5 scale; where 0=no rot and 5=plant dead. Resistance was characterized using an analysis of variance (Vanderplank, 1984). A highly significant main effect for both varieties and isolates was detected. This indicated a highly significant difference in horizontal resistance between varieties and in aggressiveness between the isolates. However, the interaction varieties x isolates was insignificant, indicating no evidence of vertical resistance.

A662

EFFECT OF ARTIFICIAL INOCULATION ON THE DEVELOPMENT OF PHOMA BLACK STEM AND PREMATURE RIPENING OF SUNFLOWER. M.L. Carson, formerly Plant Science Dept., South Dakota State University, Brookings, now USDA-ARS, Dept. Plant Pathology, North Carolina State University. Raleigh 27695-7616.

The effects of injection of hybrid sunflower stems with a conidial suspension of Phoma macdonaldii (Pm) 2 wk prior to anthesis, at anthesis, and 2 wk post-anthesis were evaluated over 3 yrs at Brookings, SD. Inoculation with Pm consistently produced greater external symptoms and internal stem decay when compared to uninoculated and sterile water injected control treatments. Precent prematurely ripened plants was greater in Pm inoculated than the uninoculated control plots. Inoculation with Pm had no significant effect on either seed yield or oil content, but 100 seed weight was significantly reduced by inoculation with Pm 2 wk post-anthesis. Percent prematurely ripened plants was significantly correlated with both external and internal symptoms as well as negatively correlated with seed yields. One hundred seed weights were negatively correlated with external but not internal symptoms. These results support the hypothesis that Pm is a factor in premature ripening of sunflower, but also suggest that other factors may be involved and that losses to Pm are slight.

A663

EFFECT OF CALCIUM SULFATE ON POD ROT OF PEANUT INCITED BY PYTHIUM MYRIOTYLUM. T. E. Clemente and A. Filonow, Departments of Plant Pathology, North Carolina State University, Raleigh, N. C. 27695 and Oklahoma State University, Stillwater, OK 74078.

Calcium sulfate was evaluated for reducing pod rot of peanut in the greenhouse and in field microplots. Sand/vermiculite mix in the greenhouse or fumigated soil in microplots were non-infested or infested with 10 or 50 propagules of P. myriotylum/g. At early bloom $CaSO_4$ was applied at 0, 560 and 1,120 kg/ha to the cv. Early Bunch in the greenhouse or at 0, 1,120 and 2,240 kg/ha to the cvs. Florunner and Spanco in microplots. At harvest, P. myriotylum was consistently associated with pod rot. Calcium sulfate was generally ineffective (P=.05) in reducing pod rot at 10 or 50 propagules of P. myriotylum/g. There were no significant (P=.05) linear correlations between calcium contents of hulls and pod rot severity. Florunner generally had more pod rot and less calcium in hulls than Spanco. Calcium sulfate rates had no effect (P=.05) on the final populations of P. myriotylum in infested mix or soil.

A666

EVALUATION OF POTASSIUM FERTILIZER, METAXYL FUNGICIDE AND SOYBEAN VARIETIES ON SOYBEAN PRODUCTION IN MINNESOTA. Ward C. Stienstra and George Rehm, Depts. of Plant Pathology and Soil Science, University of Minnesota, St. Paul 55108.

Phytophthora Root Rot (PRR) is a serious and expanding disease problem in Minnesota. Potassium fertilizer has been shown to help crops withstand root disease. The objectives of this study were to measure the effects of: 1 fertilizer K (0-0-60) rate, 2 soybean variety, and 3 fungicide (Ridomil 5G) on soybean production. All sites, 3/yr for two years had a known history of damage caused by PRR. Three factors were combined into a complete factorial using a split, split plot design with four replications. Rates of applied K (0,40,80,160lb/acre) were the main plots. Soybean varieties (II-54-254, BSR 101, Corsoy 79) were the sub plots and

Ridomil 5G (6 Lb product/ acre in furrow) or none were the sub-sub plots. K fertilizer had no measurable effect. Incidences and severity of PRR was highly dependent on variety used and environment. Fungicide use had a positive effect on yield when PRR was present. PRR incidence and severity was highly dependent on rainfall following planting.

A667

RESISTANCE TO BENZIMIDAZOLE FUNGICIDES IN *PSEUDOCERCOSPORELLA HERPOTRICHOIDES* IN WASHINGTON AND OREGON. T. D. Murray[a], R. W. Smiley[b], and W. Uddin[b], [a]Department of Plant Pathology, Washington State University, Pullman, WA 99164, and [b]Columbia Basin ARC, Oregon State University, Pendleton, OR 97801.

Mycelia of *P. herpotrichoides* were isolated on water agar + 100 µg/ml rifampin from 62 wheat samples with symptoms of eyespot. Pure cultures were maintained on potato dextrose agar (PDA), then transferred to PDA amended with Benomyl, Thiobendazole, Thiophanate-methyl, Propiconazole, Flusilazole, or prochloraz at concentrations (a.i.) of 3, 3, 4.5, 10.6, 5.1, and 5.1 µg/ml, respectively. Of 275 total isolates collected, 108 isolates from 9 fields, 3 in Oregon and 6 in Washington, were resistant to the benzimidazole fungicides. None of the isolates were able to grow on PDA amended with the demethylation inhibitors. Cropping practices in fields with resistant isolates ranged from 10-yrs continuous wheat under irrigation to 2-3 yr rotations of wheat in dryland production: all fields with resistant isolates had received 5-8 applications of a benzimidazole fungicide for control of eyespot since 1977.

A668

EFFECT OF FUNGICIDAL SEED TREATMENTS ON COMMON ROOT ROT OF SPRING WHEAT AND BARLEY. Robert W. Stack, Dept. Plant Path., North Dakota State Univ., Fargo 58105.

Common root rot, caused by *Cochliobolus sativus*, is a widespread problem on spring wheat and barley. Crop rotation and partially resistant cultivars have been the main controls but some newer fungicides have potential to control root rot when applied as seed treatments. Five seed treatment fungicides were compared in replicated trials planted on land known to have high inoculum levels of *C. sativus*. Treatment with nuarimol and imazalil reduced root rot disease rating (DR) but yield responses were inconsistent. Triadimenol also reduced root rot DR and on barley gave an 8% average yield increase in four years of trials. The subcrown internode index was a poor predictor of yield response and its suitability as a disease measurement in evaluation of seed treatments for control of common root rot is questioned.

A669

EFFECT OF TIME OF FUNGICIDE APPLICATION ON SHEATH BLIGHT CONTROL IN RICE. D.E. Groth, Rice Research Station, La. Agri. Exp. Stn., L.S.U. Agricultural Center, P.O. Box 1429, Crowley, LA 70527-1429.

Experimental plots of the rice cultivar Lemont were inoculated with Rhizoctonia solani (AG-1A). Propiconazol, benomyl and iprodione fungicides were applied with a CO_2 backpack sprayer delivering 95 l/ha at the panicle 2 mm, booting, and/or 70 percent heading stages of growth at 8:00, 10:00, 12:00, 14:00, 16:00, and 18:00 hours at labeled rates. Plots were rated for disease development at maturity and harvested. Fungicides significantly reduced sheath blight and increased yields. Time of fungicide application did not affect sheath blight control but yields were significantly reduced compared to the unsprayed control at certain timings. The only weather factor that appeared to affect fungicide performance was rainfall. Rainfall before or after spraying reduced yield increases due to fungicide application.

A671

FAILURE OF ALTERNATIVE FUNGICIDE REGIME TO DELAY DICARBOXIMIDE RESISTANCE IN BOTRYTIS CINEREA. R.J. Vali and G.W. Moorman, Dept. Plant Pathology, Penn State University, Univ. Park, PA 16802.

Strains of *Botrytis cinerea* resistant to the dicarboximide vinclozolin were detected in 80% of greenhouses surveyed in Pennsylvania, indicating the need for alternative fungicide regimes for effective disease control. Fungicide regimes were evaluated using a leaf disc assay which quantified disease incidence (# discs infected) and percent vinclozolin resistant conidia. One hundred percent resistance to vinclozolin was detected after 1 application of vinclozolin, with a concomitant loss of disease control. Disease control with the non-systemics, chlorothalonil and copper hydroxide, was 72% and 50%, respectively. Neither of these fungicides selected for vinclozolin resistance. Rotations, or full or half-strength mixtures of vinclozolin, with either non-systemic failed to delay the development of resistance. In all cases, disease control was characteristic of the non-systemic companion fungicide. In the rotations, the level of vinclozolin resistance did not decrease when the non-systemic was applied, suggesting that vinclozolin resistant populations are stable. Stability of the resistant strain in the absence of vinclozolin may be partially due to the similarity between the two strains for three of five fitness parameters evaluated.

A672

TEMPORAL DYNAMICS OF CHLOROTHALONIL RESIDUES ON PEANUT FOLIAGE. V. J. Elliott and H. W. Spurr Jr. USDA-ARS-SAA Crops Research Lab, Oxford, NC and Department of Plant Pathology, North Carolina State University, Raleigh.

Field studies were conducted to determine the persistence of chlorothalonil residues on the foliage of peanut (*Arachis hypogaea*) cv. Florigiant. Bravo 720 (formulated at 720g/l, Fermenta Plant Protection Co. Mentor, OH) was applied at a rate of 1.5 pints per acre in 40 gal of water per acre using a hand drawn plot sprayer operated with D3-25 nozzles at 40 psi. Upper canopy leaves were sampled at increasing times after application by taking 10, 1.1cm disks per sample. Five replicate samples were taken at each time period. Leaf disks were washed in toluene to remove residues and chlorothalonil levels were quantified using electron capture gas chromatography. An exponential decay model reasonably described the decline in chlorothalonil residues over time. The half-life varied between 13 and 17 days. Variation in half-life values was correlated to rainfall rates during each experiment.

A673

QUANTITATIVE STUDIES ON THE EFFECT OF BYDV-PAV AND PUCCINIA CORONATA F. SP. AVENAE ON YIELD OF TWO SPRING OAT VARIETIES. S.M. Bissonnette, C.J. D'Arcy, and W.L. Pedersen, Dept. of Plant Pathology, Univ. of Illinois, Urbana, Illinois 61801

The effects of simultaneous infection by BYDV-PAV and Puccinia coronata f. sp. avenae (P.c.a.) on the yield and yield components of two spring oat varieties were examined in 1989. The objective of this field study was to develop an empirical croploss model. Two varieties Noble (susc. P.c.a., intol. BYDV) and Ogle (susc. P.c.a., tol. BYDV), were used in the study. All plots were inoculated with BYDV-PAV by application of viruliferous aphids (Rhopalosiphum padi). P.c.a. treatments were a combination of two disease levels, and three fungicide levels (Dithane M-45). The experiment was repeated at two locations. Disease severity, yield, # florets and seeds per head, % oil, % protein and seed coat integrity were determined. Crown rust severity was assessed on several dates. BYDV-PAV incidence was determined using ELISA. The results of a second croploss study of P.c.a. infection alone also will be reported.

A674

YIELD REDUCTION OF DRY EDIBLE BEANS BY ROOT-KNOT NEMATODES IN THE CAUCA VALLEY, COLOMBIA. B.A. Mullin, G.S. Abawi, M.A. Pastor-Corrales, and J.L. Kornegay. Dept. Plant Pathology, Cornell Univ., Geneva, NY, USA, and Bean Program, CIAT, Cali, Colombia.

Tomato (cv Rutgers) seedlings infected with Meloidogyne incognita and M. javanica (RKN) were transplanted at a high density to raised beds in the trial site. Tomato foliage was removed 3 mo. later and the plot area was planted to RKN-susceptible bean cvs, which were removed at the time of trial establishment. A border of Crotalaria spectabilis was established on three sides of the infested area, and furrow irrigation was supplied on the open side. The RKN-susceptible cv. Calima was planted in the infested beds and uninfested beds outside the Crotalaria border at 15 seeds/m. Each treatment plot was 4 rows, 4-m-long,

and replicated 4X. Dry seed weight was 147 and 398 kg/ha for the RKN-infested and uninfested plots, respectively, indicating a significant 63% yield reduction ($P<0.01$) due to RKN. Similar results were obtained when the trial was repeated using the bean cv. Calima and CIAT bean line PVA 916.

A675

NUCLEUS NUMBER IN FUSARIUM CONIDIA. L. J. Stepanek and J. E. Partridge. Dept. of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722.

Using fluoresence microscopy and the DNA-binding stain 4', 6'-diamidino-2-phenylindole (DAPI), the number of nuclei per conidium was compared in cultures grown on liquid and solid (agar) media. A minimum of ten isolates each of nine species (F. moniliforme, F. subglutinans, F. proliferatum, F. anthophilum, F. dlamini, F. nygamai, F. napiforme, F. beomiforme, and F. oxysporum) were examined. The nucleus number of a minimum of 1000 conidia per isolate in both liquid and solid medium was assessed in each replicate. Harvested conidia were fixed in Carnoy's solution, rinsed in phosphate buffer, stained for 5 minutes in DAPI, rinsed, suspended and examined. The range of conidia with multiple nuclei was from <.3% to >15%. Conidia with multiple nuclei from solid media did not exceed 4% whereas their conidial equivalents from liquid were normally above 4%. The number of nuclei/conidium was more isolate rather than species dependent.

A676

DIFFERENTIATING BETWEEN MYCOSPHAERELLA TASSIANA AND THE APPLE SCAB PATHOGEN, VENTURIA INAEQUALIS ON OVERWINTERING APPLE LEAVES. C. M. Becker*, K. M. Shotwell**, S. V. Thomson**, and T. J. Burr. Cornell University*, NYSAES, Geneva, NY 14456, and Utah State University**, Logan, UT 84322.

M. tassiana (de Not.) Johans. (MT), with a Cladosporium herbarum (Pers.) Link ex Gray anamorph, was detected in overwintered apple scab infected leaves from Utah. Many morphological characteristics are similar to V. inaequalis (VI), and when both fungi were present it was tedious to determine the maturity of the VI ascospores. We present the following comparisons of both fungi to minimize confusion. MT and VI pseudothecia are similar size, but in MT they lack setae and pseudopharaphyses. Asci of MT are saccate verses cylindrical for VI, and are about one-third as numerous. Ascospores of both fungi have two, unequal-size cells, but in MT they are 20-28u long and hyaline, instead of ~14u long and colored as in VI. Mature MT ascospores can germinate within the pseudothecium, grow through the ostiole, and produce conidiophores bearing the anamorph. No disease developed when mature apples were inoculated with mycelium and conidia of C. herbarum.

A677

Biology of the dogwood anthracnose fungus, *Discula* spp.: 2. Effect(s) of pH on pathogen growth *in vitro*. Brown, D.A., R.N. Trigiano, & S.M. Twigg, University of Tennessee, Knoxville, TN 37901.

An association between dogwood anthracnose severity and acid rain has been suggested. Also, tolerance to extreme acidity for germinating *Discula* conidia has been described. Complete liquid medium (CM) was amended with HCl to pH 2.5, 3.5, 4.5, 5.5, and 6.2. Growth of *Discula* isolates from GA, MA, NY, and TN was monitored over this pH range for a 14-day period. Growth of the GA isolate (GA-1) was limited at pH 2.5 with 4 mg hyphae (dry weight) harvested. Isolate GA-1 growth increased as pH increased, to a maximum of 51mg at pH 6.2. Based upon this preliminary study, *Discula* isolates do not appear unusually tolerant of increased hydrogen ion concentrations *in vitro*.

A678

COMPARISON OF SCLEROTIAL DEVELOPMENT AMONG ANASTOMOSIS GROUPS OF *RHIZOCTONIA SOLANI* KUHN. C.S. KOUSIK AND J.P. SNOW. Department of Plant Pathology and Crop Physiology, La. Ag. Expt. Sta., La State Univ. Ag. Center, Baton Rouge, LA 70803.

Morphology of sclerotial development was studied in *Rhizoctonia solani* Kühn among anastomosis groups (AG) 2,3,4,5,6,7,8,9 and AG-BI (Bridging isolates). Samples were periodically taken from isolates belonging to the various anastomosis groups growing on PDA and were processed for scanning electron microscopy. The development of sclerotia in AGs 2,3,4,5,6,8 and 9 was from a lateral "trunk" hypha and the sclerotia were made up of monilioid cells developing from the trunk hypha. This form of sclerotial development was referred to as the lateral type. In AG-7 and AG-BI, sclerotia developed as interwoven loose hyphae and monilioid cells within the loose hyphae. Monilioid cells were observed in all the AGs studied and dense material binding the monilioid cells was also detected.

A679

PRODUCTION OF A SELF-INHIBITOR BY COLLETOTRICHUM GRAMINICOLA: SIGNIFICANCE TO SURVIVAL. B. Leite and R.L. Nicholson, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN. 47907

The conidial mucilage of Colletotrichum graminicola contained a low molecular weight material that inhibited conidial germination. The inhibitory material was water soluble and extractable into aqueous solvents by partitioning mucilage against organic solvents. Production of the inhibitor depended in part on the stage of conidium maturation. Conidia required ca. 3 days maturation after their formation in order to germinate. Depending on the substratum, conidia either formed germ tubes or appressoria, and this was influenced by conidium concentration. At high concentrations of conidia germination was inhibited completely. As concentrations were reduced, germination occurred first by appressorium formation and then by germ tube formation. Appressorium formation and/or germ tube formation was completely prevented by a partially purified inhibitor preparation, regardless of conidium age. The results indicate that a self-inhibitor is present in conidial mucilage and leaches from conidia, suggesting that the inhibitor prevents conidia from germination under adverse conditions.

A680

IMPROVED MEDIA FOR TESTING THE USTILAGO HORDEI MATING REACTION. Alfredo D. Martinez-Espinoza, Michael E. Bjarko, and John E. Sherwood. Dept. of Plant Pathology, Montana State University, Bozeman 59717.

Mating of U. hordei sporidia, which is controlled by a single locus with two alleles, results in the formation of dikaryotic mycelium which is pathogenic on barley. Ex planta mycelium formation can be used to determine the mating-type of unknowns and for complementation analysis of mutants. Several media and growth conditions were analyzed to optimize dikaryon formation and stability following mating. The addition of 1% activated charcoal to Holliday's complete or minimal agar or to Vogel's agar enhanced the intensity and stability of the mating reaction, and decreased the incubation time before mycelium was observable. Mycelial growth was transient at 20 C or 25 C, but generally stable at 6 C or 16 C. The nuclear condition of yeast and mycelial cells was confirmed by fluorescence microscopy.

A681 Withdrawn

A682

GENETIC DIVERSITY WITHIN POPULATIONS OF *FUSARIUM* SECTION *LISEOLA* FROM CORN AND SORGHUM IN KANSAS. C. Chaisrisook and J. F. Leslie. Dept. of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan, Kansas 66506-5502.

We examined 359 isolates belonging to *Fusarium* section *Liseola* recovered from separate plants at 32 sites in Kansas for mating-type and vegetative compatibility group (VCG) phenotypes. Members of all six known mating populations (A-F) were identified in this set of isolates. From corn tissue, 4 isolates were analyzed from each of 13 sites and at least 60 isolates were analyzed from each of two sites. Among isolates recovered from corn, members of the A and D populations accounted for 50-70% of the total population although there was some variation from site to site. From sorghum tissue, 4 isolates were analyzed from each of 15 sites and at least 60 isolates were analyzed from each of two sites. Among isolates recovered from sorghum, members of the D and F populations were the most common. More than 50% of the isolates from the sites with 60 isolates per site belonged to the F mating population. In the total population, the distribution of the mating type alleles was not skewed, although some local perturbations were found. Thus, the possibility for sexual genetic exchange under field conditions exists. Based on preliminary data, the relative number of VCGs appears to be quite large.

A683

CRYOGENIC PRESERVATION OF RHIZOCTONIA CULTURES. B. D. Nelson and I. Kural, Dept. of Plant Pathology, North Dakota State Univ., Fargo, ND 58105.

Mycelium of Rhizoctonia solani and binucleate Rhizoctonia spp. on potato dextrose agar (PDA) can be maintained for extended periods in cryogenic storage at -80 C. Blocks of PDA (10X4mm) with hyphae from 6-12 day old cultures were placed in sterile, polypropylene cryogenic vials (12.5 x 41 mm) with 1 ml of 10% sterile glycerol. Vials were then cooled at 4 C for 24 hr followed by 24 hr at -15 C and then stored at -80 C in an ultrafreezer. To initiate cultures from cryogenic storage, vials were immersed in water at 35 C until the ice had dissipated, then the PDA blocks plus mycelium were immediately

transferred to fresh PDA. Growth was observed within 24-48 hr at 22 C. Eleven anastomosis groups of R. solani plus 13 anastomosis groups of binucleate Rhizoctonia spp. were successfully maintained from 4-24 months in cryogenic storage. Rhizoctonia solani also survived in infected soybean stems stored at -80 C.

A684

VEGETATIVE COMPATIBILITY GROUP DIVERSITY WITHIN POPULATIONS OF *FUSARIUM MONILIFORME* ISOLATED FROM CORN SEED. R. Farrokhi-Nejad and J. F. Leslie. Dept. of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan, Kansas 66506-5502.

Fusarium moniliforme was isolated from corn seed from two cultivars both grown at four locations in the North Central United States. From the eight seed lots 123 isolates of *F. moniliforme* were recovered. *nit* mutants were generated in each isolate and used to force heterokaryons to determine vegetative compatibility. Within a seed lot between 45% and 80% of the isolates belonged to a unique vegetative compatibility group (VCG), with an average of 60%. At two sites, isolates belonging to a common VCG were recovered from each of the two cultivars. Of 74 VCGs identified, only three were present at more than one site. Of these three, one was found at three sites and the other two at two sites each. Isolates belonging to the most frequent VCG were present at a frequency of 47% within their seed lot, but only 6% within the population as a whole. These data suggest that populations of *F. moniliforme* are very localized and are genetically diverse, and that seed corn movement could provide a mechanism to explain the variability observed in commercial fields.

A685

APPRESSORIUM DEVELOPMENT IN *UROMYCES*: VESICLE AND MICROTUBULE DISTRIBUTION. Y.H. Kwon and H. C. Hoch, Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456.

Uromyces appendiculatus uredospore germlings initiate appressorium development in response to surface features (e.g., artificial ridges on plastic substrates, host stomata) by switching from polarized to non-polarized (swelling) growth. Distribution of apical vesicles and microtubules during appressorium development were determined for germlings grown on polycarbonate substrates bearing inductive ridges that trigger appressorium initiation. The germlings were examined through a 12 min time course following contact with the ridge. Serial longitudinal sections through non-differentiating germlings revealed that most apical vesicles were located within 2 μm of the substratum, and 4 μm distal to the apex. The majority of cytoskeletal microtubules were found nearest the substrate and were oriented in longitudinal profiles parallel to the direction of germling growth. During early appressorium development (4 min after ridge contact) the vesicles became redistributed at sites nearest the ridge in actively swelling regions of the apex. Microtubules in the actively swelling region were likely associated with apical vesicles, whereas those microtubules in other regions of the developing appressorium exhibited longitudinal profiles, especially over the ridge. These changes in vesicle and microtubule distribution indicate that signal reception for appressorium initiation occurred within 4 min of ridge contact. Because the vesicles were always located in the region of apical growth or swelling, they are likely involved in the expansion of the germling apex. The microtubules may be involved in vesicle transport to the new regions of growth.

A686

SEQUENCE HOMOLOGY BETWEEN NEUROSPORA CRASSA MATING TYPE GENES AND DNA FROM OTHER ASCOMYCETES. C.R. Cisar, D.O. TeBeest and F.W. Spiegel, Departments of Plant Pathology and Botany and Microbiology, University of Arkansas, Fayetteville, AR 72701.

Heterothallism in ascomycetes increases the likelihood of outcrossing and has been reported in numerous taxa. Whether this theoretically advantageous mode of reproduction has evolved once in the history of the group or several times from homothallic ancestors is unclear. Starting with the assumption that the commonly found, one locus, two allele mating type system (+/- or A/a) is homologous for many ascomycetes, the recently isolated mating type genes from Neurospora crassa (Glass, N.L. et al. Science, 1988, 241: 570) were used to probe Southern blots containing genomic DNA from a wide taxonomic range of heterothallic ascomycetes. Some of these ascomycetes show homology with the Neurospora mating type genes. These results may be useful for understanding the evolution of sexual systems in ascomycetes and for determining the phylogeny of the group.

A687

SUDDEN DEATH SYNDROME: SCANNING ELECTRON AND LIGHT MICROSCOPIC OBSERVATIONS OF SOYBEAN ROOTS INOCULATED WITH FUSARIUM SOLANI FORM A. K. S. McLean, G. W. Lawrence, and K. W. Roy, Dept. of Plant Pathology and Weed Science, Mississippi State University, Mississippi State, MS 39762.

Soybean roots inoculated in vitro with Fusarium solani form A (FS-A) were examined by scanning electron (SEM) and light microscopy (LM) to observe pathogenesis. Tissue was collected from tap roots at 4-hour intervals and prepared by standard fixation procedures. Conidia on root surfaces germinated with one or two germ tubes within 4 hours. Eight hours after inoculation hyphae and germ tubes were observed to have entered roots by direct penetration. After 24 hours, hyphae were observed growing intercellular within epidermal and cortical tissues. Epidermal and cortical cells in the infected region began to collapse 48 hours after inoculation. Intracellular hyphae were found within the cortical tissue after cell degradation occurred. Chlamydospores of FS-A were observed within the cortex.

A688

REACTION OF TALL FESCUE CULTIVARS INOCULATED WITH STEM RUST. R. E. Welty and R. E. Barker, USDA ARS, NFSPRC, Corvallis, Oregon 97331-7102.

Twenty cultivars of tall fescue were inoculated with urediniospores of stem rust. Five-wk-old seedlings (2-3 leaves on 2-3 tillers) were inoculated and scored 14 da later (3 reps, 14 plants ea). Foliage was removed and 10-wk-old seedlings re-inoculated and scored 14 da later (5 reps, 14 plants ea). Pustule types (0 & 1 = resistant; 2, 3, or 4 = susceptible) were used to calculate % resistant plants and a Disease Severity Index of 1-5 (DSI). Resistant plants as 5-wk-old seedlings ranged from 0% for Apache, Fawn, G1-307, Monarch, Maximize, Rebel II, and Shortstop (DSI ranged 4.72-4.98) to 19% for K-31, Finelawn I, and Mesa (DSI range 3.76-4.14). Resistant plants as 10-wk-old seedlings ranged from 0% for Cimmaron and G1-307 (DSI 4.61 & 4.99) to 26% for Arid and KY-31 (DSI 3.71 & 3.76). A highly significant ($P < 0.01$) difference in DSI among cultivars occurred, but all cultivars were considered susceptible to stem rust; 39 stem-rust-resistant plants were saved as a source of germplasm.

A689

FUNGI ISOLATED FROM WHITE LUPINS IN MINNESOTA. R.A. Kalis, E.L. Stewart, and R.A. Meronuck, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Lupinus albus (white lupin) is currently being grown on a limited acreage in Minnesota as an alternative crop for a source of animal protein and for human consumption. Potential fungal pathogens were surveyed on experimental and production fields in north central Minnesota where the majority of lupins are being grown. Alternaria alternata, Ascochyta sp., Fusarium acuminatum, F. avenaceum, F. moniliforme, F. oxysporum, F. solani, F. subglutinans, Pleiochaeta setosa, Pythium rostratum, P. ultimum, Rhizoctonia solani, and Sclerotinia sp. were isolated from diseased and apparently healthy lupins. Potentially the most important pathogens appear to be Ascochyta sp., Fusarium avenaceum, F. oxysporum, and Pleiochaeta setosa based on frequency of isolation and disease symptoms. Koch's postulates were conducted on isolates of the fungal species collected.

A690

OCCURRENCE OF CLAVICEPS PURPUREA AND HYPERPARASITES ON BERMUDA GRASS (CYNODON DACTYLON) AND OLD WORLD BLUESTEM (BOTHRIOCHLOA SPP.) K. E. Conway and C. M. Taliaferro, Depts. of Plant Path. and Agronomy, Oklahoma State Univ., Stillwater, OK 74078.

Production of range, pasture and turf grass seed in the Great Plains has increased as marginal lands are being removed from wheat production. In Oklahoma there are approximately 121,500 ha of seed production and may reach 405,000 ha. Heavy honey dew production by C. purpurea (Cp) has occurred in Oklahoma and Texas on old world bluestem (OWB) each year since 1986. An OWB seed sample from Roscoe, TX contained 13 to 22% sclerotial contamination. Honeydew occurred each year on bermudagrass (BG) but only at Stillwater. This is the first report of Cp on BG. Sclerotia were easier to observe in OWB because seed is larger than BG. Fruiting bodies of Cp were collected from beneath OWB in Stillwater, but not from BG. Ergot occurred on OWB and BG in both early summer and fall seed production, but was more prevalent in the fall. Two hyperparasitic fungi were recorded on the honeydew stage of Cp. Fusarium heterosporum was common on both and Cerebella andropogonis was on OWB.

A691

INFECTION PERIOD AND SITE OF INFECTION OF ANISOGRAMMA ANOMALA ON CORYLUS AVELLANA. J.K. Stone, K.B. Johnson, and J.W. Pscheidt. Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331.

Between 1 Feb - 30 April, 1988 and 2 Dec - 26 April, 1989, 13 sets of thirty 2-yr old hazelnut trees were serially exposed to natural inoculum of A. anomala within a diseased orchard. Individual sets of trees were exposed for 1 wk, replaced with a new set, and then incubated in an isolated area until symptoms developed (14-16 mo). Only trees exposed after bud break developed symptoms. In 1988, 64% of trees exposed between 14 March and 4 April, and 32% of trees exposed after April 4 became diseased. In a related study, 3 sets of 10

trees were serially inoculated with an ascospore suspension applied to opening buds and young shoots at weekly intervals between 4 - 25 April, 1988. Of trees inoculated, 80% developed symptoms. The natural infection period for A. anomala appears to coincide with bud break and early shoot elongation.

A692

PRODUCTION OF HUITLACOCHE, USTILAGO MAYDIS. J. K. Pataky Dept. of Plant Pathology, Univ. of Illinois, Urbana, IL 61801.

Common smut, caused by Ustilago maydis, is a devastating disease of sweet corn (Zea mays); but in Mexico, smut galls on ears are an edible delicacy known as huitlacoche. Recently, huitlacoche has been marketed as "maize mushroom" in the U.S. Buyers in New York City paid growers $0.50/ear for sweet corn with large ear galls. Huitlacoche is harvested about 1 wk prior to sweet corn for fresh market. To commercially produce huitlacoche, ear galls must be induced consistently. Sweet corn hybrids with greater than 40% incidence of ear galls from natural infection were identified and crossed to create a population from which to select for increased susceptibility to U. maydis. In 1989, stalk injection and leaf whorl spray inoculation procedures induced only 4% and 0.4% incidence of ear galls, respectively, and 49% and 4% incidence of stalk or leaf galls. Plant growth stage affected the host tissues on which galls formed. For example, naturally-infected plants of 'Candy Bar' planted 17 May had 41% and 9% ear and stalk galls, respectively, compared with 0% and 24% when planted 29 May.

A693

ISOLATION AND PATHOGENICITY OF BOTRYTIS CINEREA ASSOCIATED WITH CLADODE ROT OF PRICKLY PEARS (OPUNTIA SPECIES). J. O. KUTI, Horticulture Research Lab., College of Agriculture, Texas A&I University, Kingsville, Texas 78363.

A field survey for pathogenic mycoflora of naturally infected stems (cladodes) of economically important prickly pear germplasm at Texas A&I University farm was made over two years. Seven species of fungi were frequently isolated. Botrytis cinerea accounted for 25% of the isolates; species of Colletotrichum and Glomerella accounted for 16%, Aspergillus 15%, Phytopthora 11% and Macrophomina and Sclerotium 9%. This is the first record of B. cinerea as a pathogen of prickly pear. Pathogenicity of the B. cinerea isolate was tested on 25 accessions in 8 Opuntia species under greenhouse conditions. Fifteen accessions were found to be susceptible or highly susceptible while 10 accessions were resistant or highly resistant. Symptoms of pathogenicity of B. cinerea on the Opuntia species include soft rot and discoloration of affected tissues.

A694

PHYTOTOXICITY OF CULTURE FILTRATES FROM FUSARIUM SOLANI ISOLATED FROM SOYBEAN. S. M. Lim, H. S. Song, and L. E. Gray, USDA-ARS, Department of Plant Pathology, Univ. of Illinois, Urbana, IL 61801.

Culture filtrates from isolates of Fusarium solani pathogenic to soybeans were phytotoxic to soybean callus, cotyledons, and germinating seeds. Culture filtrates of the fungus grown in a synthetic culture medium for 3 weeks at 24 C caused browning in soybean, maize, tobacco, carrot and cotton callus indicating that the toxin produced by F. solani is not a host-specific pathotoxin. Drops of the culture filtrates on wounded soybean cotyledons produced chlorosis 4 days after incubation at 24 C. The root growth of germinating seeds of soybean and maize was inhibited by the culture filtrates. Variation in responses of soybean callus and cotyledons to culture filtrates was observed among fungal isolates and soybean cultivars. Ethyl acetate extracts of the culture filtrate were not phytotoxic and the extracts were negative to known T-2 toxins indicating that T-2 toxin may not be involved in the development of soybean sudden death syndrome.

A695

PARTIAL CHARACTERIZATION OF A HOST-SPECIFIC PATHOTOXIN FROM CULTURE FILTRATES OF SEPTORIA GLYCINES. H. S. Song, S. M. Lim, and J. M. Clark, Jr., Department of Plant Pathology and USDA-ARS, University of Illinois, IL 61801.

A host-specific pathotoxin isolated from culture filtrates of Septoria glycines is an autoclave-stabile, anionic, water-soluble, high molecular weight substance(s) causing typical disease symptoms of brown spot on cotyledons and leaves of soybean. The toxin was purified by sequential use of CM-cellulose treatment, DEAE-cellulose chromatography, dialysis, gel filtration, and 5% charcoal treatment. The molecular weight of the pathotoxin is approximately 20,000. Drying the toxin under flash-evaporation at 46°C in vacuo destroys more than 99% of the toxin activity. Partial acid hydrolysis with 1 N HCl at 90°C for 3 hr does not abolish the toxicity. Toxin activity is destroyed by periodate oxidation. Incubation of toxin with α-mannosidase, β-galactosidase, and β-glucosidase also reduces activity. These results indicate that the toxin contains polysaccharide, a glycosyl component is essential for the activity, and mannose, galactose, and glucose may be an essential portion of the toxin.

A696

PRODUCTION OF PERITHECIA IN NECTRIA HAEMATOCOCCA MPVI, PATHOGENIC ON PEA. P.S. Dyer. Botany School, Cambridge University, Downing Site, Cambridge, CB2 3EA, England.

The influence of light intensity on the production of perithecia in Nectria haematococca MPVI, a broad host range pathogen and the perfect state of Fusarium solani f.sp. pisi, was investigated. Four isolates were grown under different regimes of light and dark, both before and after crossing. Cool white flourescent tubes were used as a light source of varying intensity 20-150 µmol $m^{-2}s^{-1}$. Cultures were incubated at 21°C. A marked variation in perithecia production with post-spermatization light intensity was found in three isolates. Maximum numbers of perithecia were formed at light levels 20-40 µmol $m^{-2}s^{-1}$. In addition, incubation in the dark prior to crossing significantly increased perithecia production in these three isolates.

A697

EFFECT OF SUPPLEMENTAL CALCIUM ON DECAY OF APPLE CAUSED BY GLOMERELLA CINGULATA. W. S. Conway, Hort. Crops Quality Lab., Beltsville, MD 20705, C. E. Sams, Univ. of Tennessee, Knoxville, TN 37996, and J. A. Abbott, Instr. and Sensing Lab, Beltsville, MD 20705.

Apples were pressure infiltrated at harvest with solutions of $CaCl_2$ to determine the effect increased tissue calcium content has on decay caused by Glomerella cingulata. The fruit were inoculated with a conidial suspension of G. cingulata following 6 months storage at 0 C. Analysis of fruit tissue calcium indicated that a 4% solution of $CaCl_2$, pressure infiltrated into the fruit, resulted in a fruit calcium concentration of approximately 1000 ug/g dry weight. Decay caused by G. cingulata was reduced by approximately 60%. In previous work, fruit having a similar calcium content which were inoculated with Penicillium expansum had only about 30% less decay than nontreated fruit. Increased tissue calcium may differentially inhibit tissue macereation by pectic enzyme activity of postharvest decay fungi.

A699

INFECTION OF SOYBEAN ROOTS WITH SCLEROTIA-FORMING ISOLATES OF COLLETOTRICHUM TRUNCATUM, Mahmood Khan, R. E. Wagner, and J. B. Sinclair, Dept. of Plant Pathology, University of Illinois at Urbana-Champaign, Urbana, IL 61801-4709.

The pathogenicity of three sclerotia-forming isolates and one nonsclerotia-forming isolate of Colletotrichum truncatum (Ct), cause of soybean foliar anthracnose, were compared on the taproot of soybean plants cv. Corsoy using aeroponic culture at 25C. Plants were inoculated by dipping roots in a conidial suspension ($2.2x10^4$) or by attaching to the roots a piece of agar-infiltrated foam colonized by the fungus. One wk after inoculation, a 5-cm section of taproot was removed and placed on moist filter paper in a sterile culture plate. After 1.0 wk at 25C, the aver number of acervuli of Ct ranged from 25-50/root. Sclerotia-forming isolates produced more acervuli than the non-sclerotia-forming isolate. Acervuli number was the highest on roots inoculated using the foam colonized with Ct. This is the first report of Ct causing disease on soybean roots.

A700

SELECTIVE MEDIUM FOR NITRATE NONUTILIZING MUTANTS OF *FUSARIUM OXYSPORUM*. K. F. Toth and M. L. Lacy. Dept. of Botany & Plant Pathology, Michigan State University, East Lansing, MI 48824.

An agar medium was developed which slows the growth of *F. oxysporum* f. sp. *apii* race 2 wild-type (WT) strains but not chlorate-tolerant nitrate-nonutilizing mutants (*nits*) of those strains. The medium (PCA+) consisted of 500 ml potato-carrot broth (filtrate from 20 g each of carrots and potatoes autoclaved for 30 min in 500 ml H_2O), 500 ml H_2O, 40 g $KClO_3$, 0.8 g PCNB, 0.25 g chloramphenicol, and 15 g agar. PCA+ was steamed for 1 hr, dispensed, and was tested by scraping a sterile needle across colonies growing on other media, stab-inoculating plates of PCA+ 4-5 times, and measuring colony size after 5 days. The mean colony diameters from *nits* were 1.3-5.9 X larger than colonies from WT strains. Colonies grown from suspensions of conidia and chlamydospores of *nits* had diameters of 0.2-3 cm after 5 days on PCA+; the colony diameters from WT strains were all <0.2 cm. PCA+ is being tested for its ability to enumerate populations of *nits* added to soils.

A702

SENSITIVITY OF TELIOSPORES OF TILLETIA TRITICI AND T. CONTROVERSA TO METHYL BROMIDE. J. L. Smilanick, P. L. Hartsell, D. J. Henson, B. J. Goates, J. D. McKinney, and J. C. Tebbets. USDA, ARS, PWA, HCRL, 2021 South Peach Avenue, Fresno, CA 93727

Teliospores and sori of Tilletia tritici (Tt) and T. controversa (Tc) were mixed with wheat seeds (cvs. Daws and Itana) and fumigated 24 hr at 24-27C with methyl bromide (MB). The wheat moisture content (MC) was 11% or 15% before use. After fumigation, the teliospores were incubated at 20C 1 wk or 5C 8 wk for Tt and Tc, respectively. Tc was about twice as resistant to MB as Tt. Sori did not protect teliospores from MB. Results from both wheat cvs. were similar. MC greatly influenced MB activity. On wheat of 11% MC, ED95 (± 95% CI) MB dosages (g/cu m) to prevent germination were 161 (127,238) and 320 (279,473) for Tt and Tc, respectively, while on wheat of 15% MC, ED95 (± 95% CI) MB dosages were 25 (20,30) and 58 (46,111), respectively. Since high MB dosages and high wheat MC were needed for efficacy, the practicality of MB to decontaminate Tc-containing wheat is questionable for plant quarantine purposes. Repeated fumigations, vacuum fumigations, or other fumigants may hold promise.

A703

ASSOCIATION OF KERNEL SPLITTING WITH KERNEL AND EAR ROTS OF CORN IN A COMMERCIAL HYBRID GROWN IN THE COASTAL BEND OF TEXAS. G.N. Odvody, J.C. Remmers, and N.M. Spencer. Texas A&M Expt. Station, Corpus Christi, TX 78410.

In 1985, over extensive acreage in the Coastal Bend of Texas, a commercial corn hybrid developed a high incidence of ear rots caused predominantly by *Fusarium moniliforme* and less by *Aspergillus* spp. Kernel breakage approached 28% from combine harvesting and aflatoxin levels were up to 50 ppb. Mixed infections occurred but most ears were infected by only one fungus. Colonization occurred initially through lateral splits in the pericarp on the embryo side of the seed and often progressed to the whole ear. Some splits were too superficial to allow fungal colonization. The syndrome was repeated naturally in the field and the greatest incidence was associated with high yield potential and favorable early moisture followed by drought stress during later stages of grain fill. An open husk character probably contributed to entrance of fungi into ears. Other hybrids, with either parent of the susceptible hybrid represented in the cross, were not vulnerable to the kernel splitting. Only one other commonly grown commercial hybrid showed limited susceptibility to the pericarp splitting.

A704

VIRULENCE AND RACE FREQUENCIES OF PUCCINIA CORONATA IN CANADA DURING 1974-1989. J. Chong and J.A. Kolmer. Agriculture Canada, 195 Dafoe Road, Winnipeg, R3T 2M9

The changes in virulence and race frequencies of Puccinia coronata f.sp. avenae in Canada since 1974 were examined. To 1986, the eastern and prairie rust populations were dominated by avirulent and simple races. Since 1987, races with virulence to Pc39 predominated in the east. During 1974-1989 in the east, virulence frequency to Pc35 fluctuated at levels 22-53%, to Pc56 at levels 9-42%, and to Pc40, Pc46, and Pc50 at levels 1-24%. During the same period in the prairie region, virulence frequency to Pc35 fluctuated 20-51%, to Pc40 at levels 24-60%, while virulence to Pc46 increased from 6% to 72%. Gene Pc39 has been used in the east since 1983. Virulence to this gene was first detected in 1985 and increased to 87% by 1988. Cultivars with Pc38 and Pc39 have been grown in the prairie region since 1982. Virulence to this gene combination was first detected in 1987 and increased to 22% by 1989.

A705

AGGRESSIVENESS OF *PYRENOPHORA TRITICI-REPENTIS* FROM BARLEY. J.M. Krupinsky, USDA, Agriculture Research Service, Northern Great Plains Research Laboratory, P.O. Box 459, Mandan, ND 58554.

Twenty-six isolates of *Pyrenophora tritici-repentis* obtained from diseased barley leaves collected in Montana, North Dakota, and South Dakota were tested for aggressiveness. Detached seedling leaves of wheat (BH1146, Len, ND495, Red Chief, Tam 105, and Waldron) on benzimidazole agar were inoculated to compare isolates in 13 studies. Percentage necrosis of leaves was visually estimated and lesion length was measured. All isolates caused symptoms on wheat. Isolates that caused the highest or lowest percentage necrosis and lesion length symptoms and were statistically different from other isolates in a study, were designated high or low aggressive isolates. Considering the severity of symptoms, high and low aggressive isolates were comparable to the high and low aggressive isolates from wheat and other grasses. When isolates were randomly selected for comparison, all but one isolate x cultivar interaction were nonsignificant.

A706

A CHARACTERIZATION OF FUSARIA ASSOCIATED WITH CORN, COTTON, AND SORGHUM IN SOUTH TEXAS. A.S. Ring and G.N. Odvody, Texas A&M Exp. Station, Route 2, Box 589, Corpus Christi, TX 78410.

Numbers and species of Fusaria from corn, cotton, and sorghum leaves, roots, inflorescences, stems, and rhizosphere soil grown in a farming systems crop rotation experiment, Corpus Christi, Texas, were determined during crop development. Mean numbers of Fusaria were from highest to lowest on corn tassels; corn and sorghum leaves, roots, and soil; sorghum seed; and cotton tissues and soil. Numbers of Fusaria increased with crop maturation on corn and sorghum, decreased in soil, and remained low on cotton tissues. *Fusarium* species isolated were: *F. solani*, *F. equiseti*, *F. semitectum*, *Fusarium* spp. section *Liseola*, *F. oxysporum*, and *F. chlamydosporum*. Predominant Fusaria were *F. solani* and *F. equiseti* below-ground, and *Fusarium* spp. section *Liseola* and *F. semitectum* above-ground. Corn and sorghum tissues contained large numbers of *Fusarium* spp. section *Liseola* spp., whereas cotton did not contain large numbers of any Fusaria. Overall relative percentages of *Fusarium* species did not change during maturation.

A708

TRANSFORMATION AND COTRANSFORMATION OF THE TAKE-ALL FUNGUS, GAEUMANNOMYCES GRAMINIS, TO PHLEOMYCIN RESISTANCE. Alice L. Pilgeram and Joan M. Henson. Depts. of Plant Pathology and Microbiology, Montana State University, Bozeman 59717.

Gaeumannomyces graminis var. graminis and G. g. tritici (the take-all fungus) were transformed to phleomycin resistance by an improved transformation procedure with pAN8-1, a plasmid encoding the ble gene of Streptoalloteichus hindustanus. G. graminis var. graminis was cotransformed with pAN8-1 and pBT3, a plasmid encoding resistance to benomyl. Vector DNA apparently was integrated into the fungal genome in all transformants analyzed, at different sites in the genome and with varying copy numbers. The selected phenotypes ($Phleo^R$ or Ben^R) were stable through mitosis in most transformants. Integrated plasmid DNA was stable through meiosis in all transformants tested.

A709

GENETIC ANALYSIS OF STALK QUALITY TRAITS IN A MAIZE SYNTHETIC.
M.M. Held*, M.L. Carson, and Z.W. Wicks III. *Plant Science Dept., South Dakota State University, Brookings 57007; USDA-ARS, Dept. Plant Pathology, North Carolina State University, Raleigh 27695-7616.**

Two hundred North Carolina Design I progenies were produced by crossing 50 male plants with four female plants each in the South Dakota Plant Pathology Synthetic (SDPPS). Progenies were evaluated over two years at two locations for rind strength (RST) rind thickness (RT), nodal plate thickness (NT), internode length (IL), ear height (EHT), stalk lodging (SL), stalk cross-sectional area (SA), Diplodia stalk rot reaction (DSR) and grain yield (YLD). Significant additive genetic variance was present in SDPPS for all traits except RST. Dominance genetic variance was not important for any of the traits measured including YLD. SL was significantly negatively genetically correlated with RST and RT, but was not correlated with DSR or any of the other stalk traits. Selection for SL per se appears to be the best means of improving SDPPS for lodging resistance.

A710

EVOLUTION OF DISTINCT POPULATIONS OF PUCCINIA RECONDITA IN CANADA
J.A. Kolmer, Agriculture Canada, Winnipeg, MB, R3T 2M9

In Canada the eastern and prairie populations of Puccinia recondita had similar identities and frequencies of Unified Numeration (UN) races during 1931-1937 when susceptible cultivars were grown in both regions. The release of wheat cultivars with genes Lr14a and Lr3 in the prairie region in 1937 and 1943 was followed by the first directional virulence shift in the prairie leaf rust population. The continuing use of resistant cultivars in the prairie region has exerted a continuous selection pressure on the corresponding virulences in the prairie leaf rust population. Susceptible cultivars continued to be grown in the eastern region, resulting in a different succession of UN races. The current distinct regional populations most likely originated from a common introduced population of P. recondita and evolved through differences in the use of resistant cultivars.

A711

VERIFICATION OF GENES HYPOTHESIZED FOR RESISTANCE TO LEAF RUST IN WHEAT CULTIVARS OF SOUTH DAKOTA.
S.S.A. Rizvi, G.W. Buchenau, and F.A. Cholick, Plant Science department, South Dakota State University, Brookings, SD 57007.

Various spring and winter wheat cultivars with gene(s) previously hypothesized for resistance to leaf rust, were crossed to each of Leaf rust(Lr) near isogenic line for low reaction to Puccinia recondita tritici. Failure of F_2 plants from such crosses to segregate verified presence of hypothesized genes and proved the validity of analytic method. Verified genes were: A99AR,Apex83,Challenger:Lr 1; Butte,Norseman,Wheaton, Oslo,Norak and Olaf:Lr 10; Erik,Marshall and Len: Lr 2a.Lr 10; Butte86:Lr 10.Lr 24; Guard and Shield: Lr 2a.Lr 3.Lr 10; Bennet,Brule,Lancer,Rita and Rose: Lr 3; Dawn,Nell:Lr 3.Lr 10; Centura and Sage:Lr3.Lr24 and finally Siouxland:Lr 3.Lr 24.Lr 26.

A712

EFFECTS OF WHEAT LEAF PUBESCENCE ON INFECTION BY Puccinia recondita. J. J. Roberts, D. L. Long, R. E. Wilkinson and G. G. Ahlstrand, USDA/ARS, Cereal Rust Laboratory, St. Paul, MN 55108 and The University of Georgia, Griffin, GA 30223.

Scanning electron photomicrographs of densely pubescent wheat leaves inoculated with urediniospores of Puccinia recondita f. sp. tritici provided evidence that infection was influenced by leaf hairs. Spores were frequently trapped by hairs, "stranding" them above the leaf surface. Contact with leaf hairs disrupted normal germ tube growth along the leaf surface often resulting in failure to find a stoma and infect. Subsequent light-microscope observations of inoculated seedlings provided further evidence of erratic growth patterns by leaf rust germ tubes and reductions in the number of germinating spores which initiate infection. Over 90% of germ tubes observed on cv. Hunter's glabrous leaves exhibited normal growth whereas 40% were normal on Combo's densely pubescent leaves. Slight reductions in infection in each disease cycle can significantly slow epidemics of leaf rust. Therefore leaf pubescence may provide an important level of protection.

A713

COMPARISON OF MYCOSPHAERELLA SPECIES IN WHEAT STUBBLE. R. B. Madariaga and D. G. Gilchrist. Department of Plant Pathology, University of California, Davis, CA 95616

Two species of Mycosphaerella occur on wheat stubble in California. They produce morphological similar hyaline didymoascospores but differ in their biology and are connected to different anamorphs. The ascospores of one species measure 18-20 µm and on water agar germinate by germ tubes which develop into typical fruiting structures of Cladosporium herbarum, a cause of the Black Point disease of wheat. Its teleomorph is M. tassiana. In the second species the ascospores measure 13-16 µm; they produce only blastospores which are indistinguishable from blastospores formed by pycnidiopores of Septoria tritici the causal agent of Septoria Leaf Blotch of wheat.

A714

EFFECTS OF TRICHODERMA HARZIANUM ON REPRODUCTION OF MELOIDOGYNE SPECIES ON TRIFOLIUM REPENS. G. L. Windham and M. T. Windham, USDA-ARS, Mississippi State, MS, 39762; and Dept. of Entomology and Plant Pathology, Univ. of Tennessee, Knoxville, TN 37901.

The effect of Trichoderma harzianum (TH) isolate T-12 on the reproduction of Meloidogyne incognita (MI) and M. arenaria (MA) on Trifolium repens cultivar Regal (nematode susceptible) and germplasm SC-1 (nematode tolerant) was determined in greenhouse studies. A peat-wheat bran inoculum of TH was added to soil prior to transplanting clover seedlings into 10-cm pots. MI or MA were added at transplanting at a rate of 5000 eggs per pot. MI reproduction was significantly ($P = 0.05$) lower on Regal and SC-1 grown in TH treated soils. MI reproduction was reduced by 38% on Regal and 58% on SC-1. TH had less of an effect on MA, with reproduction reduced by 8% on Regal and 29% on SC-1.

A715

DISTRIBUTION AND INCIDENCE OF HETERODERA GLYCINES IN IOWA SOYBEAN FIELDS. L. E. Sweets and N. K. Baker. Iowa State University, Ames, IA 50011.

A study to monitor the distribution of Heterodera glycines (SCN) within production fields and the changes in population levels of SCN with various crop rotations within those fields was initiated in 1988. A grid pattern of sampling sites was laid out through each field using permanent markers so that the same site could be sampled annually. Soil samples have been taken from all sampling sites in all fields each spring prior to or immediately after planting. A 100 cm^3 subsample of each sample was processed for eggs of H. glycines using a mechanical extraction method. SCN was found to be widely distributed in all of the fields sampled. Levels (based on SCN egg counts) within an individual field varied greatly (ex. 100 eggs/100 cm^3 soil to 11,100 eggs/100 cm^3 soil). One year of SCN susceptible soybeans lead to a significant increase in SCN levels within monitored fields.

A716

BEHAVIOR OF *CRICONEMELLA XENOPLAX* ON ROOTS IN MONOXENIC CULTURE. S. W. Westcott, III, Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634.

Criconemella xenoplax (Raski) Luc and Raski was perpetuated in monoxenic culture on root explants of *Trifolium incarnatum* L. 'Dixie' by repeated subculture. The medium contained Gamborg's B-5 salts (Exp. Cell Res. 50:151-158. 1968.). Second stage juveniles (J-2) frequently accumulated in these cultures (46% of population at 6 wk) and a low proportion (4%) of the nematode population was observed feeding at any one time. On roots from cuttings of *Prunus besseyi* L. H. Bailey

the proportion of J-2 in the population was lower (35%) and more nematodes fed at once (13%) than on *T. incarnatum*. *Criconemella xenoplax* could not be perpetuated by subculture on seedling roots of *Prunus persica* L. 'Nemaguard' or *Dianthus caryophyllus* L. 'Double Grenadin', or root explants of *Zea mays* L. 'Golden Sweet Bantam', *Lycopersicon esculentum* Miller 'Rutgers', or *Cucumis sativus* L. 'National Pickling'. Adults fed and subsequently laid eggs, but the J-2 accumulated in cultures; up to 80% of the population were J-2 on roots of *L. esculentum* and *D. caryophyllus* roots after 7 wk. Few eggs were laid and juveniles did not develop on the other plants.

A717

EFFECTS OF YELLOW NUTSEDGE (CYPERUS ESCULENTUS) AND PURPLE NUTSEDGE (C. ROTUNDUS)ON MELOIDOGYNE INCOGNITA POPULATIONS IN CHILE PEPPERS (CAPSICUM ANNUUM). S.H. Thomas and J. Schroeder, Department of Entomology, Plant Pathology and Weed Science, New Mexico State University, Las Cruces, NM 88003.

Root-knot nematodes (M. incognita host race 3) and both yellow and purple nutsedge are severe and frequently concomitant pests of chile peppers in New Mexico. The effects of different pest combinations on plant growth parameters and nematode populations were investigated in a factorially designed greenhouse experiment. Nematode numbers (infective J2) were 50% less in pots containing chile and either weed species. Both C. esculentus and C. rotundus were hosts of M. incognita, but egg production per gram of root tissue was less than 3% of that observed on chile roots. Such information on the effects of root competition between weed and crop species on population dynamics of plant-parasitic nematodes is needed in the development of representative pest management models.

A718

CORN-COTTON ROTATIONS FOR THE MANAGEMENT OF THE RENIFORM NEMATODE. G. W. Lawrence, G. L. Windham*, K. S. McLean, W. E. Batson, Jr., and J. C. Borbon, Dept. of Plant Pathology and Weed Science and USDA-ARS*, Mississippi State University, Mississippi State, MS 39762.

Corn (Zea mays) and aldicarb were evaluated for the management of Rotylenchulus reniformis in cotton (Gossypium hirsutum) production in Mississippi. Corn (Pioneer Brand 3165) and cotton (Deltapine 20) were planted in a field previously planted to cotton and naturally infested with a high population of R. reniformis. Each crop was planted alone and with aldicarb (Temik 15G) at 1.18 kg ai/ha. R. reniformis population densities at harvest were significantly larger in plots where cotton was planted without aldicarb, with a reproductive factor (RF) of 5.3. The lowest R. reniformis populations were recovered from the corn plots with an average (RF) of 0.07. Corn cultivar Pioneer Brand 3165 does not appear to be a host for R. reniformis and may be a useful rotation crop in cotton production systems.

A719

EFFECT OF PROLONGED EXPOSURES TO ROOT LEACHATES FROM RESISTANT AND SUSCEPTIBLE SOYBEAN CULTIVARS ON HATCH AND EMERGENCE OF HETERODERA GLYCINES. E. J. Sikora and G. R. Noel. USDA-ARS, Dept. of Plant Pathology, Univ. of Illinois, Urbana, IL 61801.

Egg hatch and emergence were determined for second-stage juveniles (J2) of Heterodera glycines races 3 and 4 exposed to soybean root leachate of cv. Fayette (resistant to H. glycines) and cvs. Asgrow 2575, Asgrow 3127, and Williams 82 (susceptible to H. glycines) over a 52-d period. Leachate was obtained from plants at 4-d intervals beginning 7 d after planting using double-distilled water. Twenty cysts from field-grown soybeans were incubated in 2 ml of leachate at 25 C. At 4-d intervals, emerged J2 were counted and removed and leachate from 4-d older plants was added. Leachate obtained from Asgrow 2575 stimulated more hatch (88 and 72%) and emergence (86 and 65%) of race 3 and 4, respectively than did leachate from the other cultivars. Leachate obtained from Fayette stimulated less hatch (25 and 28%) and emergence (5 and 8%) of race 3 and 4, respectively. Hatch and emergence was greatest during the initial 12 days of the experiment.

A720

NEMATODE ASSOCIATIONS WITH OAK DECLINE. G. W. Lawrence, V. Ammon, K. S. McLean, J. Tate, *T. E. Nebeker, *F. I. McCracken, and *J. D. Solomon. Dept. of Plant Pathology and Weed Science, Mississippi State University, Miss. St., MS 39762 and *Southern Hardwoods Lab., USDA, Stoneville, MS.

Nematodes were extracted from soil taken from oak decline and control sites at seven locations within the Missisippi and Tennessee-Tombigbee River basins. Soil was collected from trees with decline and trees with no evidence of decline. Nematode communities were separated into tropic groups based on characteristics of the stomodoeum. A total nematode population density of 526 and 651 nematodes/500 cm^3 of soil were recovered from decline and healthy trees, respectively. Stylet bearing nematodes were recovered in highest frequency (56%), followed by fungivors (26%), bacterivors (14%) and predators (4%). Fifteen species of stylet bearing nematodes were recovered. Nematodes included Tylenchus spp. (47%), in highest frequency, Xenocriconemella sp. (24%), Criconemella sp. (9%), Meloidogyne sp. (11%), Helicotylenchus sp. (7%) and Dolichodorus sp. (2%).

A721

APPLICATION OF CLONED DNA FRAGMENTS TO DIFFERENTIATE AND DETECT *PERONOSCLEROSPORA* SPECIES. C. L. Yao, C. W. Magill, R. A. Frederiksen, and *M. R. Bonde. Dept. of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843 and *USDA-ARS, Frederick, MD, 21701.

P. sorghi, *P. sorghi* (Thailand isolate), *P. maydis*, *P. philippinensis* and *P. sacchari* were readily distinguished by the EcoRI RFLP patterns produced when six radioactively labeled probes were hybridized to Southern blots containing DNA from the respective fungi. The patterns were the same whether DNA was extracted directly from the fungus or from infected leaves. No intraspecific RFLPs were detected in limited tests. The probes were selected from a *P. sorghi* pathotype 3 DNA library in pUC 19. Sorghum downy mildew and Java downy mildew inoculum in sorghum seeds and maize seeds collected from plants infected with *P. sorghi* and *P. maydis* respectively, were detected by probe pMLY 12 which contains 1.5 and 1.3 kb DNA fragments. This probe did not hybridize to DNA extracted from 10 of the common fungi of sorghum and maize, or to DNA isolated from plant tissue infected with other downy mildews, *Sclerospora graminicola* or *Sclerophthora macrospora*.

A722

THE IDENTIFICATION AND CHARACTERIZATION OF A RACE 3-SPECIFIC REGION FROM *PSEUDOMONAS SOLANACEARUM*. Douglas Cook and Luis Sequeira, University of Wisconsin-Madison, Madison WI 53706.

Race 3 strains have the unique ability to cause wilting of potatoes at low temperatures and represent the most homogeneous group within *Pseudomonas solanacearum*. Members of race 3 have remarkably similar RFLP patterns and are thought to have evolved in geographical isolation in the Andean region of South America, although strains have been disseminated world wide via infected potato tubers. By means of subtractive hybridization, we have identified a 2 Kb cosmid clone that has homology with all race 3 strains tested, but not with other members of the species. Overlapping cosmid clones with homology to the original race-specific clone were obtained from a race 3 gene library; subsequent analysis of these clones has established that the race specific region is comprised of two clusters containing at least 26 Kb of DNA.

A723

DETECTION OF PHYTOPHTHORA CINNAMOMI ON AZALEA WITH ELISA. D. M. Benson, Dept. Plant Path., N. C. State Univ., Raleigh, NC 27695.

Hinodegiri azalea growing in pine bark:sand (3:1) in a container nursery were inoculated with *P. cinnamomi* at either 0, 1, 3, 9, 30, or 90 colonized oat grains/plant. One wk after introduction of *P. cinnamomi* and then at 2-wk intervals for the next 13 wk, root samples were collected from each plant. Each sample was divided into two sub-samples with one cultured on PPP medium and the other assayed by ELISA in a multiwell kit-E (Agri-Diagnostics Assoc., Cinnaminson, NJ). *P. cinnamomi* was detected after 1 wk from 10% of the plants sampled by both culture and ELISA methods. After 3 wk, detection was 45% and 35% for culture and ELISA, respectively. Detection by ELISA reached 50% by 7 wk, and 90% by 13 wk. The number of culture-ELISA positives was consistent beginning at 3 wk and thereafter. No correlation was found between inoculum rate and absorbance. Cross-reaction was not apparent. Comparable results were found in a subsequent experiment in the greenhouse with a rapid assay kit. ELISA was a reliable method for detection of *P. cinnamomi*.

A724

INDOLEACETIC ACID ENHANCEMENT OF C^{14} METHIONINE UPTAKE BY TOMATOE CELL CULTURES AIDS ASSAY OF PATHOGEN EXTRACTS. Clarence Madhosingh, London Research Centre, Agriculture Canada, 1400 Western Road, London, Ontario, Canada, N6G 2V4.

One micromolar indoleacetic acid (IAA) produced a six-fold increase in three hours in C^{14} methionine uptake by washed tomatoe cells in suspension. This system allowed a practical differentiation of pathogen (Fusarium oxysporum radicis lycopersici) and non-pathogen (F. oxysporum) materials used for treating the cells, based on C^{14} methionine uptake. This IAA effect facilitates the development of a rapid assay system for Fusarium wilt pathogens of tomatoes.

A725

MONOCLONAL ANTIBODIES TO A SALINE EXTRACTABLE ANTIGEN ASSOCIATED WITH CONIDIA OF *PYRICULARIA ORYZAE*, RACE IB49. J. Q. Xia, L. N. Raymond, F. N. Lee and H. A. Scott. Hybridoma Laboratory and Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Two mouse monoclonal antibody-producing hybridomas, 2B5A1 and 2D1F1, were developed using germinated conidia of *Pyricularia oryzae* Race IB49 as the immunogen. Monoclonal antibodies produced by both hybrids reacted in an enzyme immunoassay and an immunofluorescent assay (IFA) with a phosphate-buffered saline extractable substance associated with *P. oryzae* conidia, and normal and infected rice leaf tissue, and rice seed. IFA with ascites produced by hybrid 2B5A1 also resulted in identification of the same or a cross-reactive epitope on conidia of *Aspergillus* sp., *Penicillium* sp., and five isolates of *Pyricularia grisea*. No reactivity with *Alternaria sp.*, *Curvularia* sp., or *Fusarium* sp. conidia was observed. IFA indicated that the epitope is localized on the surface of both *P. oryzae* conidia and conidiophores but not on germ tubes.

A726

USE OF IMMUNOGOLD LABELLING WITH SCANNING ELECTRON MICROSCOPY TO DETECT BACTERIA ON LEAF SURFACES. C. L. Davis, R. H. Brlansky and D. S. Howd. University of Florida, CREC, Lake Alfred 33850.

Scanning electron microscopy coupled with backscattered electron (BSE) imaging was used to detect colloidal gold-labelled specific immunoglobulins (IgG) attached to epiphytic bacteria. Greenhouse grown Citrus paradisi Macf. cv. 'Duncan' plants were spray inoculated with 0.5×10^8 cfu/ml of strains of Xanthomonas citri, X. campestris pv. citrumelo, X. campestris pv. pruni, and Erwinia herbicola. Leaf disc samples were collected at 1, 2, 3, 5, and 7 days postinoculation and fixed in glutaraldehyde/ruthenium red. Afterwards the discs were incubated on gold-labelled IgG, washed, dehydrated, and carbon coated. Strains of X. citri and X. campestris pv. citrumelo were distinguished from the other bacteria when labelled with homologous gold-labelled IgG which was viewed in the scanning electron microscope with BSE imaging. Reverse polarity was used to observe individual gold particles attached to the bacteria.

A727

SENSITIVITY OF ELISA IMMUNOASSAY KITS TO SPECIES OF PHYTOPHTHORA COLLECTED WORLDWIDE. J. W. Pscheidt, J. Burket and P. B. Hamm. Dept. Bot & Pl Path, Oregon State Univ, Corvallis, OR 97331-2903.

The sensitivity of a Phytophthora specific ELISA immunoassay (Kit E, Agri-Diagnostics, Cinnaminson, NJ) was tested on 17 species of *Phytophthora* including 18 isolates each of *P. cinnamomi* and *P. cactorum* collected throughout the world. Isolates were grown in liquid (GYP) media for 7-10 days at 19°C. A 20 mg sample of aspirated mycelia was ground in sterile sand and 2 ml 'extract solution', boiled and diluted such that 5 ug fresh wt mycelia was tested. Absorbance of the assay at 405 nm was compared to an 'extract solution' control ground in sand. All *Phytophthora* isolates produced a positive reaction with the immunoassay. The lowest absorbances relative to other species were obtained from *P. cinnamomi* and *P. megasperma* (subgroup Apple/Cherry). Variation in absorbance was high among isolates of *P. cinnamomi* but low among *P. cactorum*. The 'Kit E' immunoassay reacted to a wide range of *Phytophthora* species found worldwide, however, the sensitivity of the assay was variable among species and isolates.

A728

COMPARISON OF TOMATO RINGSPOT VIRUS DETECTION BY IMMUNOLOGICAL AND NUCLEIC ACID HYBRIDIZATION ASSAYS. **E. V. Podleckis[1,2], R. A. Owens[1], and A. Hadidi[2]. [1]USDA, ARS, Microbiology and Plant Pathology Lab., Beltsville, MD and [2]USDA, ARS, National Plant Germplasm and Quarantine Lab., Glenn Dale, MD.**

Tomato ringspot virus (TomRSV) detection by nucleic acid hybridization assays and indirect enzyme linked immunosorbent assay (I-ELISA) were compared to determine their relative sensitivities. Total nucleic acid extracts from infected plants were bound to nitrocellulose membranes and probed with radiolabeled complementary RNA or DNA probes. The 1000 nucleotide cRNA riboprobe detected amounts of TomRSV RNA equivalent to picogram quantities of intact virions. Synthetic cDNA oligoprobes, 15-20 nucleotides long and I-ELISA of equivalent dilutions of crude sap from TomRSV-infected plants were 100 and 1000-fold less sensitive, respectively.

A729

ELISA MICROTITER PLATE UNIFORMITY STUDY. E. M. Bauske, S. G. Carmer, A. D. Hewings, and F. L. Kolb. Dept. of Plant Pathology and USDA/ARS, University of Illinois, Urbana, Il 61801.

To characterize the ability to detect treatment differences, the variability between and within 12, Immulon 1 "U" plates was determined using an indirect monoclonal enzyme-linked immunosorbant assay for the barley yellow dwarf virus (BYDV-PAV-IL) antigen. A single sample of Clintland 64 oat tissue infected with BYDV-PAV-IL was prepared and partially clarified by centrifugation to assure uniformity. All reagents used in the assay were prepared in bulk and applied to all 12 plates. The single sample was placed in all wells and the plates were read 40 min. after the substrate was added. Plate mean absorbances ranged from 0.955 to 1.429 OD. The coefficients of variation for each plate ranged from 8.44% to 16.90%. Incomplete blocks were imposed on each plate to determine if variability within plates could be controlled through experimental design. The results did not indicate consistent control. Patterns of variability within plates and results of tests to evaluate the assumption that absorbances are normally distributed with a common variance will be discussed.

A730

GAERTNERIOMYCES SP. AS A POTENTIAL BIOLOGICAL CONTROL AGENT OF SYSTEMIC DOWNY MILDEW INFECTION OF *SORGHUM BICOLOR* (MOENCH). I. S. Kunene, G. N. Odvody, and R. A. Frederiksen, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas 77843.

Incorporation of *Gaertneriomyces* sp., a chytrid, in microplots containing soil with *Peronosclerospora sorghi* oospores reduced systemic downy mildew infection in *Sorghum bicolor* plants. Reductions in systemic infection were dependent on the amount of chytrid added. Microplots containing 13,100 cm^3 of silty clay loam soil at pH 8.1 were treated with 250 ml of chytrid (high) and 125 ml chytrid (low) at a concentration of 100 thalli/ml. Systemic infection was reduced by 42% in low concentration treated plots and by up to 58% in high concentration treatments. Spraying had no influence on levels of disease.

A731

THE ROLE OF PYOLUTEORIN AND FLUORESCENT SIDEROPHORE PRODUCTION BY PSEUDOMONAS FLUORESCENS PF5 IN BIOCONTROL OF PYTHIUM DAMPING-OFF OF COTTON. J. Kraus and J. E. Loper, USDA, ARS, HCRL, Corvallis OR 97330.

Pseudomonas fluorescens Pf5, a biocontrol agent of Pythium damping-off of cotton, produces many anti-fungal compounds in culture, including pyoluteorin (Pyo) and pyrrolnitrin, an uncharacterized antibiotic, a fluorescent siderophore (Flu), ammonia, and cyanide. Thirteen Pyo^- and 14 Flu^- mutants, with single Tn*5* insertions in 8 and 7 distinct EcoR1 fragments, respectively, were obtained. Strain Pf5 and Flu^- derivatives were antagonistic against P. ultimum on 523 medium in the presence of $FeCl_3$, while Pyo^- derivatives were not. Pf5, Flu^-, and Pyo^- strains were indistinguishable with respect to antagonism on nutrient agar supplemented with 2% glucose. Pf5, two Pyo^- mutants, and two Flu^- mutants established similar rhizosphere population sizes and were indistinguishable statistically with respect to biocontrol of cotton damping-off in a Willamette sandy loam (pH 6.0) containing indigenous Pythium spp. at approximately 5 ppg. Results suggest that biocontrol of Pythium damping-off is not mediated by pyoluteorin or fluorescent siderophore production of Pf5 in this soil.

A732

PRODUCTION OF AMMONIA BY PSEUDOMONAS CEPACIA INTERFERES WITH SEED GERMINATION AND ROOT ELONGATION. M. Baligh, K. E. Conway and M. A. Delgado, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078 and Universidad Nacional de Piura, Piura, Peru.

P. cepacia used as a biological seed treatment on vinca and sage to control soilborne diseases interferes with germination and root elongation. High concentrations of bacteria on seeds (10^6 - 10^8) interfere with germination more than lower concentrations. Germination was affected in split-half petri dishes in the presence of P. cepacia. Because no direct contact occurred this indicated that a volatile compound was involved. pH paper suspended over bacterial cultures showed changes from 7 to 8. Cultures were grown in Czapek's broth with and without the addition of 20 g/L of peptone. Volatiles were collected in water traps and tested for the presence of ammonia using an ammonium test kit (EM Science Co., Gibbstown, NJ). Five strains of P. cepacia produced 10 to 50 ug/ml of ammonia only when peptone was added to the medium.

A733

FUNGI ASSOCIATED WITH MYCELIUM OF *RHIZOCTONIA SOLANI* IN BARE PATCHES OF WHEAT. N. Worasatit and K. Sivasithamparam. University of Western Australia , Nedlands , W.A. 6009 . Australia .

Mira cloth discs colonized by *Rhizoctonia solani* were incubated in soil samples collected from bare patch and non-bare patch areas of a wheat field to isolate fungi which were associated with the mycelia of *R. solani* . These fungi were screened for inhibition of growth of *R. solani* on agar. Twenty species/forms were encountered in non-bare patch soil while 50 species were isolated from patch soil. Most isolates belong to *Fusarium, Trichoderma, Penicillium, Chrysosporium, Mortierella* or *Phoma* . *Trichoderma spp.* were significantly more frequent within patches where they were dominant in the centre and periphery of the patches . This may be related to the recovery seen in plants remaining in the centre and the abrupt end of the patch at the periphery. *Penicillium spp.* and seven other fungi, including a *Trichoderma sp.*, inhibited growth of *R. solani* on PDA while only five isolates of *Penicillium* and one each of *Aspergillus* and *Trichoderma* showed inhibition on tap water agar.

A734

SACCHAROMYCES CEREVISIAE PROTECTS MAIZE PLANTS, UNDER GREENHOUSE CONDITIONS, AGAINST COLLETOTRICHUM GRAMINICOLA. S.F.Pascholati, S.R. da Silva*, and W.B.C. Moraes*. ESALQ/USP, P.O.Box 9, 13400-Piracicaba-S.P.; *Biological Institute, P.O.Box 7119, 01051-São Paulo-S.P., Brazil.

Suspensions from washed or non-washed S.cerevisiae cells and filtrates of these suspensions, obtained from commercial baker's yeast (CBY), reduced the development of C.graminicola as well as the expression of anthracnose on maize leaves treated with these preparations. When cells of S.cerevisiae were isolated from CBY and grown in PDA medium, the cell suspensions and their filtrates also reduced the development of C.graminicola and the expression of the disease. The yeast preparations and their filtrates were shown to be thermolabile. The reduction on the development of C.graminicola and on disease expression, when filtrates of S.cerevisiae were used, suggest that the presence of the yeast cell is not necessary to protect the leaves. In vitro experiments showed that S.cerevisiae cells exhibit a possible antagonistic activity against C.graminicola due to antibiosis.

A735

BIOLOGICAL CONTROL OF BOTRYTIS ACLADA IN ONIONS. J. Köhl, W.M.L. Molhoek, and N.J. Fokkema, Research Institute for Plant Protection (I.P.O.), 6700 GW Wageningen, The Netherlands.

In a field experiment with artificial inoculation, the effect of two strains of Trichoderma viride on the incidence of onion neck rot caused by Botrytis aclada was studied. Antagonists were applied as a conidial suspension immediately after leaf topping during the harvest procedure. Rot assessed after 3 months storage at 9°C under initially favourable conditions for fungal development was reduced from 35% to 24%. Under favourable conditions (drying at 30° for 14d after harvest) rot was reduced from 5% to 2%. A bio-assay for a rapid selection of new antagonists was developed based on the reduction of wound infection of detached onion leaves. Of the 40 isolates tested, strains of Gliocladium spp., Trichoderma spp., Penicillium spp. and Aureobasidium pullulans were superior to those used in the field. Antagonists were also selected for interference with sporulation of B. aclada on leaf debris to reduce inoculum load in the field.

A736

THE EFFECT OF SEED INOCULATION WITH SELECTED BACTERIAL STRAINS ON THE EARLY STAND COUNT OF TWO CORN HYBRIDS. D.M. Haefele, J.L. Marlow, and C.S. Stauffer. Microbial Genetics, A Division of Pioneer Hi-Bred International.,Inc., 7300 N.W. 62nd Ave., Johnston, IA 50131.

In 1989 field trials at six locations in the midwest Pioneer® Hybrids 3475 and 3585 showed significantly higher early stand counts when treated with selected bacterial inoculants than when seed was treated with water alone. Some treatment-hybrid combinations were better than or equal to Captan seed treatment. Analysis of variance showed that location, treatment, and the interaction of treatment with hybrid and with location were significant sources of variation. Early stand count of non-treated 3475 seed was improved 6.2% by Captan, 16.3% by water alone, and 21% by a mixture of 3 bacterial strains applied in water. Early stand count of non-treated 3585 seed was improved 11.4% by Captan, only 6% by water alone, and 11% by the mixture of 3 bacterial strains applied in water.

A738

Suppression of Phytophthora cinnamomi *in vitro* and *in vivo* by microbial antagonists isolated from a compost mix. M.P.You and K.Sivasithamparam, University of Western Australia, Nedlands, W.A. 6009

Four fungi and one actinomycete isolated from a potting mix containing composted Eucalypt bark were found to inhibit Phytophthora cinnamomi on agar. The actinomycete (isolate A4) was deleterious to the growth of the assay plants (Antirrhinum sp.) . In non-sterilized potting mix, all four fungi (isolates of Trichoderma , Aspergillus (2) and Humicola) reduced root rot with the Aspergillus sp. (isolate C236) being the most effective. The effect of these fungi and an antagonistic sterile red fungus on the survival and growth of the pathogen in sterilized and non-sterilized potting mix was investigated.

A739

EPIDEMIOLOGICAL AND HOST RANGE STUDIES OF PUCCINIA JACEAE, A POTENTIAL BIOCONTROL AGENT OF PURPLE STARTHISTLE. N. Shishkoff and W. L. Bruckart, USDA-ARS, Bldg. 1301, Fort Detrick, Frederick, MD 21701.

Puccinia jaceae Otth. is a rust fungus from Europe which infects Centaurea calcitrapa L. (purple starthistle), an introduced weed of California pastures. Urediniospores germinated on agar over a temperature range of 12-30 C with maximum germination after at least 8 h at 18-27 C. The greatest number of pustules developed with 8 or more hours of dew at 15-21 C. The latent period of infection was 15 days at 15 C and 9 days at 20 or 25 C. The response-surface models developed will be used to predict infection in nature using temperature and duration of dew period. Of 63 genera tested, only a few genera in the tribe Cardueae were susceptible to Puccinia jaceae. Artichoke, safflower and a few native Cirsium species developed minor infections and became less susceptible with age.

A740

INHIBITION OF PHYTOPHTHORA VIGNAE BY SOIL BACTERIA. W.G.D.Fernando and R.G.Linderman, Oregon State University and USDA-ARS Horticultural Crops Research Laboratory, Corvallis, Oregon 97330

Phytophthora vignae, cause of stem and root rot of cowpea (Vigna unguiculata), was inhibited in vitro by (1) unidentified bacteria isolated from cowpea field soils in Sri Lanka where the pathogen was present but the disease was absent, and (2) by known biocontrol agents from other sources (Pseudomonas, Enterobacter, and Bacillus). P. vignae was inhibited on undivided plates of PDA, Kings B (KB), Tryptic soy agar (TSA), corn meal agar, and nutrient agar (NA), but only on TSA and NA or KB where divided plates were used (volatile inhibitors). Volatile inhibitors were absorbed by the medium supporting the pathogen, and pH increased therein. Substrates like tryptic soy and cowpea seed extract were necessary for production of volatile inhibitors by bacteria added to sterile soil.

A742

ANTAGONISTIC MICROORGANISMS TOWARD MACROPHOMINA PHASEOLINA IN VITRO. F. Perdomo, E.C. Schroder, and R. Echávez-Badel. Departments of Agronomy & Soils and Crop Protection, University of Puerto Rico, Mayaguez Campus, Mayaguez, P.R. 00709.

Ashy Stem Blight caused by Macrophomina phaseolina has been recently reported as a severe disease of dry bean in the Caribbean region. In order to identify biological control microorganisms against M. phaseolina, we have been screening bacteria to detect potential antagonists. Three in vitro methods, the streak plate, double layer and spent culture were used to measure bacterial antibiosis towards M. phaseolina. Significant reductions in radial growth rates of M. phaseolina were found with presence of an actinomycete, Pseodomonas cepacia and Xanthomonas campestris pv. phaseoli.

A743

SELECTION OF RHIZOBACTERIA FOR BIOCONTROL OF PYTHIUM ULTIMUM ON CUCUMBER FOR GREENHOUSE APPLICATION. E.M. Tipping, S.E. Campbell, E.E. Onofriechuk, S. Young and R. Munagala.

Bacterial strains were isolated from several peat and plant-based sources using a variety of isolation methods to increase the probability of obtaining a diverse collection of organisms. A total of 514 strains were screened in repeated growth chamber assays on cucumber in sterile sand and vermiculite amended with Pythium ultimum. On the basis of consistent, superior performance 45 strains, mostly fluorescent pseudomonads isolated from indigenous bog plants, were selected for further testing. They were subsequently screened under greenhouse conditions in a commercial peat-based growing mix artificially infested with P. ultimum. Several strains elicited a final stand superior to the chemical control. In order to elucidate possible mechanisms, the elite strains were screened for in vitro antagonism to P. ultimum and other soilborne plant pathogens, cyanide production, direct growth promotion in sterile growth pouches and production of plant growth regulators.

A744

PRIMING OF PELLETIZED BIOCONTROL FUNGI FOR ENHANCED HYPHAL GROWTH AND SPORULATION. G. R. Knudsen, D. J. Eschen, and L.-M. Dandurand. Plant Pathology, PSES, University of Idaho, Moscow 83843.

Alginate pellet formulations of biocontrol fungi may control plant pathogens, weeds, and insect pests, but the time required for rehydration and subsequent hyphal outgrowth and/or sporulation may limit their efficacy under some environmental conditions. We formulated fermentor biomass of Trichoderma harzianum in alginate/bran pellets, allowed the pellets to dry only partially, primed them in a 40% aqueous solution of polyethylene glycol for 12-24 hr, then allowed them to dry. On water agar, colony radii from primed pellets were 45-98% larger after 24-48 hr (22 C) than from unprimed pellets, and conidia were produced about 48 hr sooner. Similar results were observed in steamed soil, and with the insect biocontrol fungus Beauveria bassiana.

A745

REDUCED GROWTH OF *ERYSIPHE GRAMINIS* F. SP. *HORDEI* INDUCED BY *TILLETIOPSIS PALLESCENS*. A.L. Klecan[1], S. Hippe[2], and S.C. Somerville[1,3]. [1]DOE-Plant Research Laboratory and [3]Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824, U.S.A.; and [2]Institute of Botany, Christian Albretchts University, D-2330 Kiel 1, Federal Republic of Germany.

The leaf epiphyte, *Tilletiopsis*, which possesses hyaline ballistoconidia, was found contaminating barley seedlings infected with the obligate parasite *Erysiphe graminis* f. sp. *hordei*. The contaminant was identified as an isolate of the rare *Tilletiopsis pallescens* based on morphological, physiological, and biochemical characteristics. An antagonistic relationship between *E. g. hordei* and *T. pallescens* was demonstrated on the surface of barley leaf segments. On a gross level, *T. pallescens* caused severe reduction of mycelial expansion and spore production by *E. g. hordei*, whereas *T. minor* was antagonistic to a lesser extent. Low temperature scanning and conventional transmission electron microscopy showed that hyphae of *E. g. hordei* were collapsed and degenerated in the presence of *T. pallescens*.

A746

GERMINATION RESPONSES TO VOLATILE AROMA COMPOUNDS BY TELIOSPORES OF A UROMYCES SP. FROM EUPHORBIA VIRGATA. A. R. Bennett and R. C. French, USDA-ARS, Frederick, MD 21701.

Teliospores of a Uromyces sp. were collected from Euphorbia virgata in 1989 near Stavropol, USSR for evaluation as a potential biocontrol agent for leafy spurge (Euphorbia spp.) in the U.S. To evaluate germination potential, teliospores were exposed to volatile compounds previously known to stimulate germination of other rust spores. Of 12 compounds tested, benzonitrile induced the greatest response. Germination on agar +/- 50 ul/L benzonitrile was greatest between 20 and 25 C in both light and dark. In darkness, germination without stimulator was less than 20% at the optimum temperature (ca 25 C), but increased to 60% with 50 ul/L benzonitrile. Maximum germination (80%) occurred in the light without benzonitrile. These results indicate germination is favored by light and can be enhanced in the dark by exposure to benzonitrile.

A747

CLAVIBACTER MICHIGANENSIS SUBSP. SEPEDONICUS, AN EFFECTIVE BIOCONTROL AGENT AGAINST MAJOR POSTHARVEST DISEASES OF POME FRUITS. W. Janisiewicz, USDA, ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430.

Clavibacter michiganensis subsp. sepedonicus, isolate K-5 (PFS-15-48S) was isolated from mature 'Bartlett' pear after repeated washing in phosphate buffer with mild sonication. This bacterium was a top performer among 24 most promising antagonists selected after primary screening against Penicillium expansum (incitant of blue mold) and Botrytis cinerea (incitant of gray mold) on apples and pears. In tests with various concentrations of the antagonist (5 and $1.7x10^8$ CFU/ml and $4.8x10^7$ CFU/ml), complete control of blue mold and gray mold was achieved with $5x10^8$ and $1.7x10^8$ CFU/ml on pears, and with $1.7x10^8$ and $4.8x10^7$ CFU/ml on apples, respectively. The antagonist survived well over a 30 day period (duration of the experiment) at the wound site on 'Golden Delicious' apples stored at 1C, and as a wet paste preparation at 4C.

A748

ATTACHMENT OF ANTAGONISTIC YEAST TO FRUIT ROTTING FUNGI: FURTHER CHARACTERIZATION OF BIOCONTROL ACTIVITY AND CHARACTERIZATION OF POSSIBLE INHIBITORY ACTION. C. Biles, M. Wisniewski, C. Wilson, and R. McLaughlin. USDA-ARS, AFRS, Kearneysville, WV 25430.

Yeast isolate US-7 protects apples and peaches from postharvest fruit rotting fungi (Botrytis cinerea & Penicillium expansum). In order to examine the yeast-pathogen interaction, fungi were grown on agar plates overlayed with cellophane. Yeast isolates were applied to the plates 24 hr later near the young hyphal growth. Samples were taken 24 hr later from the section where the fungi and yeast had intersected.. Light microscopy revealed a general attachment of the effective biocontrol agent US-7 and non-effective isolate 117. Scanning electron microscopy indicated that both isolates attached to the fungal hyphae, but the US-7 isolate attached more tightly. Twenty-four hours after applying the US-7 isolate to B. cinerea and P. expansum, pitting and hyphal collapse were observed. Previously the mode of antagonism was suggested to be nutrient competition. However, this research indicates other mechanisms may also play a role in biocontrol.

A749

EFFECT OF APPLE FRUIT TISSUE CALCIUM ON POSTHARVEST BIOCONTROL EFFICACY OF *CRYPTOCOCCUS LAURENTII* AGAINST *BOTRYTIS CINEREA*. R. G. Roberts and J. T. Raese, USDA, ARS, Tree Fruit Research Laboratory, Wenatchee, WA 98801.

'Golden Delicious' apple trees were sprayed to runoff four times in June, July, and August with either $CaCl_2$ at 2.4 g/L, $CaNO_3$ at 1.0 g/L, or were unsprayed (calcium controls). Fruit were harvested from all parts of the canopies of five single tree replicates per treatment, then surface disinfested by immersion in chlorine dioxide. Fruit cortex and peel tissue samples from 40 fruit per tree were pooled for mineral analysis. Ten fruit per replicate per treatment were wounded once, then 10 µl of a 10^7 cfu/ml suspension of *Cryptococcus laurentii* (RR87-108) were placed in each wound. Treated wounds were then immediately challenged by addition of 10 µl of a 2 X 10^4 conidia/ml suspension of *Botrytis cinerea*. Pathogen controls received only *Botrytis* conidial suspensions. Inoculations were repeated twice, and data from all three trials were pooled for analysis. Percentages of wounds with *Botrytis* lesions were determined after storage for 12 days at 16 C. Fruit treated with $CaCl_2$ had significantly ($p=0.05$) greater calcium content (238 ppm) than did $CaNO_3$-treated (208 ppm) or untreated (196 ppm) fruit, and had 28% less decay among the pathogen controls. Biocontrol efficacy of *C. laurentii* did not differ between the calcium treatments, however, as only one of 450 fruit treated with the yeast became infected.

A750

BIOLOGICAL CONTROL OF MUCOR ROT OF PEAR BY *CRYPTOCOCCUS LAURENTII, C. FLAVUS* AND *C. ALBIDUS*. R. G. Roberts, USDA, ARS, Tree Fruit Research Laboratory, 1104 N. Western Avenue, Wenatchee, WA 98801.

Four strains of *Cryptococcus flavus* (RR89-154, RR89-156, RR89-160, RR89-211) and one strain each of *C. albidus* (R89-212) and *C. laurentii* (RR89-129) isolated from pear leaves and fruit gave effective biological control of Mucor rot of pear fruit. Biocontrol efficacy of these strains against Mucor rot was evaluated in ripe and non-ripe 'Anjou' pears by treating artificial wounds with 10 µl of 10^8 cfu/ml buffer-washed yeast cell suspensions, then immediately challenging the wounds with 10 µl of a 10^5 spore/ml suspension of *Mucor piriformis*. Control wounds received only buffer and *Mucor* spores. Ten fruit per each of four reps per yeast strain were stored for 5-12 days at 5, 10, or 15 C, then percentages of wounds with lesions were determined. Each experiment was repeated once. Reduction in percentages of wounds in treated fruit that became infected relative to controls in ripe pears varied with incubation temperature and yeast strain. The ranges and means (in parentheses) of percentage reductions in ripe pears from all trials were; 12.8-(22.7)-35.9 at 15 C, 17.5-(49.6)-60.0 at 10 C, and 37.5-(81.7)-95.0 at 5 C. In non-ripe pears, percent reductions were 87.0-(94.0)-100.0 at 15 C, 97.6-(98.8)-100.0 at 10 C, and 100% for all strains at 5 C. Repeated trials gave similar results.

A752

PROTECTION OF COTTON SEEDLINGS AGAINST RHIZOCTONIA SOLANI AND PYTHIUM SPP. BY BACTERIAL SEED TREATMENT. M.L. Courtney and J.C. Rupe. Department of Plant Pathology, University of Arkansas, Fayetteville, AR. 72701.

Bacteria were isolated from the hypocotyls of healthy cotton seedlings grown in soil naturally infested with pathogenic Pythium spp. and Rhizoctonia solani. Cotton seeds were treated with each of 139 bacterial isolates and planted in naturally infested soil. Pots were incubated at 21 C for 14 days. Seedling mortality was measured, and seven isolates improved stands significantly ($p < 0.05$) relative to nontreated controls and were not significantly different from the fungicide (PCNB) control. In field tests, one isolate (Arthrobacter globiformis) provided protection in soil where R. solani was the principal pathogen and another isolate (unidentified) provided protection in soil where Pythium spp. were the principal pathogens. No one isolate was effective against both pathogens.

A753

BIOLOGICAL CONTROL OF RHIZOCTONIA ROOT ROT OF WHEAT BY VERTICILLIUM BIGUTTATUM AND A STERILE RED FUNGUS. K.B. Cowling and K. Sivasithamparam. The University of Western Australia, Nedlands, W.A., Australia. 6009.

A Western Australian isolate of Verticillium biguttatum controlled root rot in wheat caused by a patch strain of Rhizoctonia solani Kühn. V. biguttatum reduced disease in R. solani-inoculated pots containing nutrient-poor white sand or soil from a wheat field with a history of the patch disease. Three methods of inoculating soil with a V. biguttatum spore suspension were compared: a) direct contact with R.solani inoculum for 24 hr, mixed with soil and incubated for 2 wk before planting b) mixed with soil and R. solani inoculum 2 wk before planting and c) added with seed at planting 2 wk after the soil had been infested with R. solani. V. biguttatum effectively controlled root disease in a and b, but not c, suggesting that disease control was due to pathogen suppression. A sterile red fungus from W.A. also significantly reduced Rhizoctonia root rot after 6 days of incubation with the pathogen in field soil.

A754

EFFECT OF PSEUDOMONAS FLUORESCENS ON GROWTH AND PROLIFERATION OF TRICHODERMA HARZIANUM IN STEAMED AND RAW SOIL. Li Bin, G. R. Knudsen, and D. J. Eschen. Plant Pathology, PSES, University of Idaho, Moscow, 83843.

Alginate pellets of T. harzianum (Th) were buried in steamed or raw soil (matric potential -1 or -5 bars) with P. fluorescens (Pf) strain 2-79 (at 0, 10^5, or 10^8 cfu/g of soil). Trichoderma and Pf were enumerated in both soils after 7 and 14 days; hyphal growth and density of Th were measured in steamed soil. Pf populations remained unchanged in steamed soil, and inhibited Th: mean cfu/g of Th increased from 0 to $>10^4$ with Pf absent, to 3.5 x 10^3 with 10^5 cfu/g of Pf, and to 10^3 with 10^8 cfu/g of Pf. Pf also reduced hyphal density. In raw soil, Pf declined exponentially (mean = -1.6 logs/day). Trichoderma remained at the background level of about 200 mean cfu/g whether Pf was present or not.

A755

BIOCONTROL OF RHIZOCTONIA ROOT AND CROWN ROT OF SOYBEANS BY BACILLUS MEGATERIUM ATCC-55000, Z. L. Liu and J. B. Sinclair, Dept. of Plant Pathology, University of Illinois, Urbana, 61801.

B. megaterium ATCC-55000 was studied as a potential biocontrol agent on soybeans in the field. Seeds treated with 55000 resulted in a significantly lower disease index caused by R. solani 65L-2 at 100 ug/ml soil applied in-furrow compared to Bacillus subtilis A13 and CA8. Significant (p=0.25) yield increase was recorded for 2 yr in successive treatments, but not the second after 1 yr treatment inthe presence of R. solani. A significant reduction in recovery of R. solani was obtained after in-furrow treatment with 55000. Yield increase (p=0.05) was recorded in 30-cm clay pots coptaining soil treated with 55000 + R. solani. Strain 55000 at 10^6 cfu/g soil was required for disease control in the rhizosphere. Yield from plants treated with 55000 were significantly higher than untreated control and Vitavax 200 for cv. Williams 82 at Urbana. No significant yield differences were recorded in three other field plots in heavier soils in central and northen Illinois.

A756

EVIDENCE FOR EXCHANGE OF AFLATOXIN PRECURSORS RESULTING IN AFLATOXIN PRODUCTION DURING CO-FERMENTATION OF AFLATOXIN NON-PRODUCING STRAINS OF ASPERGILLUS PARASITICUS. T. E. Cleveland, D. Bhatnagar, and R. L. Brown, USDA, ARS, Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA 70124

Aspergillus flavus strains were examined for their ability to secrete aflatoxin (AF) pathway metabolites during liquid fermentation. An A. parasiticus mutant (SRRC 163) blocked in an early step (averantin to averufin conversion) in the AF pathway retained about 80% of the total averantin produced inside the fungal mycelia. In contrast, another non-aflatoxigenic strain of A. parasiticus (SRRC 2043), which accumulates the AF pathway intermediates, O-methylsterigmatocystin (an AFB1 precursor) and dihydro-O-methylsterigmatocystin (an AFB2 precursor), secreted greater than 50% of these metabolites. Co-fermentation of these non-aflatoxigenic strains, SRRC 163 (blocked early in the pathway) and strain SRRC 2043 (blocked late in the pathway), resulted in AFB1 and AFB2 synthesis. The results indicate that, during co-fermentation, certain AF non-producing strains can secrete and exchange AF pathway metabolites and produce aflatoxins.

A757

THE EFFECT OF ULTRAVIOLET LIGHT HORMESIS ON CONTROLLING POSTHARVEST ROTS OF THREE TYPES OF FRUIT.
C. Stevens, C. L. Wilson, J. Y. Lu, V. A. Khan, M. K. Kabwe and H. Zhonk. Dept. of Agricultural Sciences, Tuskegee University, AL 36088 and USDA/ARS/NAA Appalachian Fruit Research Station, Kearneysville, WV. 25430.

Low doses of ultraviolet light (254nm UV-C) irradiation reduced postharvest rots of pome, stone and citrus fruits. Brown rot (Monilinia fructicola) of Elberta and Loring peaches was significantly reduced by UV-C 10 and 20 days respectively, after irradiation. The UV-C levels which gaved the best results for Loring and Elberta were 7.5 to $40x10^4$ ergs/mm^2 and 4.8 to $20x10^4$ erg/mm^2, respectively. Thirty days after treatment with $7.5x10^4$ erg/mm^2 of UV-C apples showed roting due to Alternaria spp M. fructicola and bacteria soft rot at 9.5, 0 and 0%, respectively; whereas, controls showed 36, 12 and 12% rots, respectively. The application of UV-C was effective in controlling green mold rot (Penicillium digitatum), stem end rot (Alternaria citri), as well as sour rot (Geotrichum candidum) of Dancy tangerines 18 days after irradiation. The optimum dosage levels for controlling sour and stem end rot were $0.8x10^4$ erg/mm^2 to $20x10^4$ erg/ mm^2.

A759

TOXICITY OF THE MYCOTOXINS FUMONISINS B_1 AND B_2 AND Alternaria alternata f. sp. lycopersici (AAL) TOXIN IN CULTURED MAMMALIAN CELL LINES. W. T. Shier, Dept. of Medicinal Chemistry and H. K. Abbas, C. J. Mirocha, Dept. of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Fumonisins B_1 and B_2 and AAL toxin are a series of structurally related mycotoxins. Fumonisins B_1 and B_2, produced by Fusarium moniliforme, induce toxic hepatitis and hepatomas in rats and leukoencephalomalacia in horses. The cancer-promotion assay which has been used to guide their purification is slow and consumes large amouunts of sample. We have examined a series of cultured mammalian cell lines in order to develop a more rapid and sensitive bioassay for use in isolation and mechanism of action studies. Of 9 rat hepatoma cell lines tested, all except the two most dedifferentiated lines were sensitive, with a toxic response visible by 48 hrs. Approximate LD_{50} values for the most sensitive hepatoma line, H4TG, were 5, 2 and 10 μg/ml for fumonisins B_1, B_2 and AAL toxin, respectively, in 100 μl cultures. Among 15 other cell lines examined, only MDCK dog kidney epithelial cells were sensitive (LD_{50} = 3, 3 and 5 μg/ml, respectively).

A760

FUMONISIN ISOLATION AND PURIFICATION FROM CULTURED CORN, AND ITS DETERMINATION IN FEED AND CORN SAMPLES. R. F. Vesonder, R. E. Peterson, J. Haliburton*, and P. A. Jursinic. Northern Regional Research Center, USDA/Agricultural Research Service, Peoria, IL 61604. *Texas A&M Veterinary Medical Diagnostic Laboratory, Amarillo, TX 79116-3200.

Fumonisin B_1 (FB_1) in corn and feed has been implicated as the cause of equine leukoencephalomalacia (ELEM) [Marasas et al, *Onderstepoort J. Vet. Res.* 1988)]. We developed a process to isolate FB_1 from corn cultured for 18-21 days at 25C with *Fusarium moniliforme*. Samples were extracted with aq. CH_3OH, water or aq. acetonitrile, and extracts were purified by XAD-2 column chromatography and high performance liquid chromatography (HPLC). FB_1 was detected either by refractive index or fluorescence emission after conversion to its o-phthalaldehyde derivative. The FB_1 (450 mg, 95% purity) was obtained by preparative HPLC from 800 g fermented corn. This method was also used to screen 22 feed and corn samples implicated in ELEM. Large concentrations of FB_1 (>100 ppm) were detected in at least six of 18 positive samples.

A761

IMMUNOASSAY FOR DETECTION OF ERGOLINE ALKALOIDS IN TALL FESCUE. B. J. Savary, B. B. Reddick, K. D. Gwinn and M. H. Collins-Shepard. University of Tennessee, Knoxville, TN 37901-1071.

Acremonium coenophialum, an endophytic fungus associated with tall fescue, produces ergoline alkaloids which may have a role in fescue toxicosis of grazing animals. The objective of this research was to develop an immunoassay for detection of these alkaloids in forage samples. Various protein conjugates of an ergonovine derivative were synthesized and used to immunize rabbits. The antiserum which was selected for further study had a high titer for binding a non-immune conjugate and was sensitive to competition by both ergonovine and ergopeptines. When used in a direct competitive ELISA, this antiserum distinguished between endophyte-infected (E+) and endophyte-free (E) tall fescue.

A762

ULTRASTRUCTURAL CHANGES IN DORMANT MONILINIA FRUCTICOLA CONIDIA WITH HEAT TREATMENT. D. A. Margosan and D. J. Phillips. USDA, ARS, PWA, HCRL, 2021 South Peach Avenue, Fresno, CA 93727

Monilinia fructicola conidia were heated in 52C water for 0, 0.5, 1.0 or 2.0 min. Germination after 24 hr on water agar was 94.8, 65.6, 2.3 and 0.3%, respectively. Earliest conidial ultrastructure damage occurred as disruption of mitochondrial cristae (0.5 min). Longer treatment times resulted in further disruption of mitochondrial cristae, matrices, and outer mitochondrial membranes, disruption of vacuolar membranes, and gaps in the conidial cytoplasm. No ultrastructural changes were observed in the nuclei or cell wall after treatment, as reported for germinated conidia. The site of heat lethality in dormant M. fructicola conidia appears to be situated in the mitochondria, probably in the inner membrane.

A763

PRODUCTION OF ERGOLINE ALKALOIDS IN BOTH LIQUID AND SOLID MEDIA BY ACREMONIUM COENOPHIALUM ISOLATED FROM TALL FESCUE SEED. K. D. Gwinn and B. J. Savary. University of Tennessee, Knoxville, TN 37901-1071.

Limited availability of ergovaline, the predominant ergopeptide alkaloid produced by Acremonium coenophialum, hinders research on its role in fescue toxicosis. Isolates of the fungus were obtained from a single seed lot and grown in both liquid fermentation and solid culture. Extracts, obtained from liquid (mycelium and filtrate) and solid cultures with 2% tartaric acid and acetone (30:70), were partially purified by liquid-liquid partition extraction. Extracts, fluorescent under long wave UV light and positive with p-dimethylaminobenzaldehyde reagent, were separated by reverse phase HPLC using fluorescence (235 excitation, 435 emission) detection. A major fluorescent peak which was observed in all extracts coeluted with authentic ergovaline.

A764

PLANT AND ANIMAL TOXICITY OF CULTURE FILTRATES OF PHOMOPSIS LONGICOLLA P1 and P12 TO SOYBEANS AND CHICKEN EMBRYOS. S. Z. Shah, Z. L. Liu, and J. B. Sinclair, Dept. of Plant Pathology, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801-4709.

Isolates P1 and P12 of Phomopsis longicolla, cause of soybean seed decay, from field-grown soybean seeds cv. Williams 82, were studied for their toxicity to soybean seedlings and embryonated chicken eggs. The toxic components of 21-day-old culture filtrates of each isolate were tolerant to heat for 10 min at 60, 75, 90, and 100C; to pH 3.4; and to intensive fluorescent light for 24 hr. Isolate P12 caused more severe cotyledonary browing than P1 to 12-day-old soybean seedlings after 2.0 days suspended in culture filtrates. Both isolates significantly inhibited germination of soybean seeds in culture and soybean root development compared to potato-dextrose broth or deionized distilled water controls. Seedlings suspended in 12% culture filtrate of each isolate in distilled water were dead in 6.0 days. Chicken embryos were killed within 6.0 days at 37C when incubated with 2.0 ml of crude culture filtrates.

A765

DO CELERY HYDROLASES PROVIDE RESISTANCE AGAINST THE FUNGAL PATHOGEN *FUSARIUM OXYSPORUM* F. SP. *APII*? S.L. Krebs and R. Grumet, Department of Horticulture, Michigan State University, East Lansing, MI 48824.

Specific activities of 2 plant hydrolytic enzymes, endochitinase (CHIT) and β-1,3-glucanase (β13G), were determined following germination of celery seeds ('FL 683') on soil containing either: a non-pathogen (*F.o.* f.sp. *cepea*), *F.o.* f.sp. *apii* race 1 (incompatible), *F.o.* f.sp. *apii* race 2 (compatible), or no inoculum (control). In all treatments except the control, CHIT activity was detected 14 days after germination (DAG), peaked at 21 DAG, and then declined. At 28 DAG, leaf yellowing and wilting was observed in the compatible interaction.

Peak CHIT activities in roots and shoots were lowest in control and non-pathogen treatments, 2X higher in the resistant interaction, and 8X higher in the susceptible interaction. β13G activity was localized in roots, and levels of induction were similar for both compatible and incompatible pathotypes (~2X over control). Treatment of celery roots with chitosan solutions (25 μg/ml) resulted in a 6X induction in CHIT activity, and 2X increase in β13G activity. Current experiments are testing whether chitosan-treated 'FL 683' seedlings show enhanced resistance to race 2 (compatible).

A766

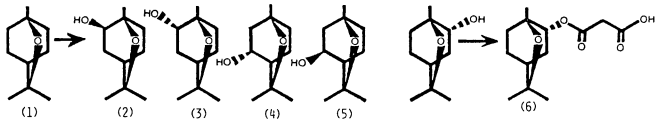
CHEMICAL ANALYSIS OF THE AROMA EMITTED BY PYCNIA OF THE CANADA THISTLE PATHOGEN, PUCCINIA PUNCTIFORMIS. R. C. French and W. J. Connick, Jr., USDA-ARS, Bldg. 1301, Ft. Detrick, Frederick, Md. 21701 and USDA-ARS, P.O. Box 19687, New Orleans, La. 70179

Pycnia of the Canada thistle pathogen, Puccinia punctiformis (Strauss) Roehl., produce a highly scented nectar which may attract insects for cross fertilization. Volatile compounds emitted from the yellow colored pycnia of systemically infected thistle shoots were collected on Tenax columns, eluted, and analyzed by GC-MS. Benzaldehyde, phenylacetaldehyde, phenethyl alcohol, and indole were identified as the predominant compounds from the rusted (flowerless) thistle shoots. Volatiles from thistle flowers contained the same compounds, minus indole. None of these compounds stimulated the germination of aeciospores or teliospores of P. punctiformis. The identified compounds may be useful in biocontrol procedures to attract insect predators to young Canada thistle [Cirsium arvense (L.) Scop.] and perhaps aid in rust dispersal.

A767

BIOTRANSFORMATION OF MONOTERPENES BY GLOMERELLA CINGULATA Mitsuo MIYAZAWA, Hiroshi NAKAOKA, Mitsuro HYAKUMACHI* and Hiromu KAMEOKA, Department of Applied Chemistry, Faculty of Science and Engineering, Kinki University, Higashiosaka-shi, Osaka, 577 JAPAN. *Lab. of Plant Disease Science, Faculty of Agriculture, Gifu University, Yanagido, Gifu, 501-11 JAPAN.

The microbial transformation of 1,8-cineol(1) and camphor(7) by Glomerella cingulata were studied. 1,8-Cineole(1) was transformed to a mixture consisting of 2-exo-hydroxy 1,8-cineole(2), 2-endo-hydroxy 1,8-cineole(3), 3-endo-hydroxy 1,8-cineole(4), 3-exo-hydroxy 1,8-cineole(5) and [1R, 2R, 4S]-2-endo-hydroxy 1,8-cineolyl maloneate(6). Pathway for oxidation of 1,8-cineole have been proposed based on the structural evidence and time course change. On the other hand, camphor(7) was transformed to a major metabolite(hydroxy camphor) by G. cingulata.



A769

ANNUAL-PERENNIAL SOMATIC HYBRIDIZATION IN MEDICAGO - CHARACTERIZATION OF HYBRID CALLI. L. B. Johnson, M. R. Thomas[1], F. F. White, and D. W. Goad, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, and [1]Division of Horticulture, CSIRO, Adelaide, South Australia 5001.

Somatic hybridization may be useful to transfer disease and insect resistance to alfalfa from annual Medicago species sexually incompatible with it. Thomas et al.(Plant Science, in press) obtained hybrid calli, embryos, and plantlets after protoplast fusion and antibiotic selection, using transformed alfalfa (kanamycin resistant) and annuals (hygromycin resistant). In further studies, only limited root formation has occurred from calli of alfalfa × M. scutellata fusions. Three of six confirmed alfalfa × M. intertexta calli from independent fusions produced embryos and roots, another only roots. Two plantlets formed from embryos of one fusion, but they were vitrified and did not live. Three plants were regenerated from a seventh fusion, but attempts to demonstrate that they are hybrids have been inconclusive. Efforts to characterize them continue.

A770

ELISA AND IMMUNOCYTOCHEMICAL DETECTION OF FUSARIUM SOLANI-PRODUCED NAPHTHOQUINONES IN CITRUS TREES IN GROVES WITH BLIGHT. S. Nemec, S. Jabaji-Hare, and P. M. Charest, USDA, ARS, Orlando, FL 32803; and Dept. Phytologie, Universite' Laval, Ste-Foy, Canada.

Xylem fluid of symptomless 1-2.5-cm dia. scaffold roots and branches of healthy-appearing and blight-diseased citrus trees in ridge and flatwoods Florida groves contained naphthoquinone toxins of F. solani by competitive ELISA analysis. Blighted tree roots contained two to five times more toxin than those of healthy-appearing trees. Concn. were as high as 100,000 ng/ml and fluctuated seasonally. Concn. in roots of trees in blight-suppressive organic flatwoods soils were less than those in roots of trees on adjacent blight-conducive sands. In F. solani-infected roots, fungal cell walls and vacuoles were labeled by colloidal gold; and intense labeling of the outer fungus wall surface suggested that toxins were secreted into the vessel lumen. The fungus probably synthesizes these toxins when it causes root rot on fibrous roots, and from there are translocated to other plant parts.

A771

PURIFICATION AND CHARACTERIZATION OF PEPTIDES FROM MAIZE SEED WHICH SHOW ANTIFUNGAL ACTIVITY. J. Duvick, T. Rood, and G. Rao. Pioneer Hi-Bred Int'l, Inc., 7300 N.W. 62nd Ave., Johnston, IA 50131

We are examining maize seeds for proteins that are inhibitory to plant pathogenic fungi. We report the identification of several small, acid-soluble, basic peptides with antimicrobial properties and comprising approx. 0.3% of the soluble protein of mature maize kernels (inbred B73). One of the peptides (CM-III) was purified to homogeneity by cation exchange FPLC. CM-III inhibits spore germination or hyphal elongation of several fungi including Fusarium graminearum and Sclerotinia sclerotiorum. It has a MW of ~4000 on SDS-PAGE, is rich in arginine and glutamine, and has no obvious sequence homology to the thionins, a conserved group of cysteine-rich peptides with antimicrobial activity found in other cereals and some dicots.

A772

ISOLATION AND CHARACTERIZATION OF *ASPERGILLUS NIDULANS* MUTANTS RESISTANT TO THE ANTI-FUNGAL COMPOUND LY214352. A. J. Smith, G. E. Davis, and G. D. Gustafson, DowElanco, P. O. Box 708, Greenfield, IN 46140.

We have isolated and characterized six chemically-induced mutants of the haploid, filamentous fungus *Aspergillus nidulans* that are resistant to the experimental fungicide 8-chloro-4-(2-chloro-4-fluorophenoxy)quinoline (LY214352). The mutants are 13- to 430-fold more resistant to LY214352 than a parental strain and one of the mutant strains requires LY214352 for maximal growth. The resistance trait is controlled by a single dominant or partially dominant gene in each mutant and it is likely that all of the mutations are allelic. The mutants were not cross-resistant to other compounds that inhibit growth of *A. nidulans*. This suggests that the mechanism of action LY214352 may be unique.

A773

OPINE-INDUCED Ti PLASMID CONJUGATION IS SUBJECT TO NEGATIVE AND POSITIVE REGULATION. S. Beck von Bodman, S.-W. Qin, S. W. Allen, and S. K. Farrand, Department of Plant Pathology, and Department of Microbiology, University of Illinois at Urbana-Champaign, Urbana, Illinois, 61801.

*Eco*RI fragment 26 of pTiC58 encodes a central repressor function that negatively coregulates expression of pTiC58 agrocinopine catabolism and agrocinopine-induced conjugal transfer. This repressor responds to crown gall-specific agrocinopine opines with the consequence of full expression of both phenotypes. Comparative DNA sequencing analysis of *Eco*RI fragment 26 from the wild-type Ti plasmid and corresponding DNA from a spontaneous constitutive mutant, pTiC58Tra^{C}, identified a five basepair deletion in the mutant genome that contributes to the constitutive phenotype. Isolation of a *Tn*5-induced Tra^{C} mutant that remains regulated for opine catabolism suggested that conjugal transfer is controlled at a second level. Specifically, the *Tn*5-specific and spontaneous Tra^{C} mutant Ti plasmids express reporter genes within Tra region II. In contrast, *Tn*5-induced Tra^{-} mutations in Tra region I fail to express these same reporter functions, thus indicating that a Tra I-specific gene product, i.e., an activator, is essential for

transcription of Tra II-related genes. Accordingly, conjugal transfer of pTiC58 is subject to primary negative regulation through an agrocinopine-sensing mechanism, and a positive secondary level of regulation through a Tra region I-encoded activator function.

A774

REGULATION OF PAPILLA FORMATION AND INDUCED CELLULAR RESISTANCE. K. Yokoyama, J. R. Aist, and C. J. Bayles, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Papillae are localized plant cell wall appositions that can stop penetration attempts by parasitic fungi. We have discovered a papilla-regulating factor (PRF) in aqueous extracts of barley leaves prepared by autoclaving. The PRF induced oversize papillae, increased papilla frequency from 65% to 95%, and reduced the penetration efficiency of the powdery mildew fungus from 80% to 2.5%, all in susceptible barley. Moreover, the PRF induced lignification in both barley and radish cell walls. Known elicitors of lignification prepared similarly from several plant sources also induced papilla formation and resistance in susceptible barley. These results suggest that the PRF is an elicitor. The PRF had no apparent, direct, deleterious effect on either the host or the parasite. We will identify the PRF and clarify the mechanism by which it regulates papilla formation.

A775

AN INHIBITOR OF PECTIN LYASE FROM SUGAR BEET. W. M. Bugbee. U.S. Department of Agriculture, Northern Crop Science Laboratory, P.O. Box 5677, Fargo, ND 58105-5677.

Pectin lyase (PNL) was the major pectolytic enzyme produced by *Rhizoctonia solani* AG 2-2 in culture and in infected sugar beet crowns and roots. A constitutive inhibitor of PNL (PNLi) was extracted from healthy sugar beet roots. The PNLi was purified by cation exchange, affinity and gel filtration chromatography. The PNLi is a protein with a molecular weight estimated at 43 kD. Inhibitory activity was most effective at pH 6.5. The average content of PNLi for crown, hypocotyl and root tissue was 40% higher in a root rot resistant germplasm line than in a susceptible cultivar and was higher in the root than the crown of the resistant cultivar. PNLi partially protected cells from damage caused by PNL. Growth of *R. solani* in liquid culture was not inhibited by PNLi.

A776

USE OF FLUORESCENT DYES TO MONITOR THE EFFECTS OF OLIGOGALACTURONIDES ON ACTIVE OXYGEN METABOLISM. E. W. Orlandi, & C. J. Baker, Department of Botany, University of Maryland, College Park, MD, 20742 and U.S.D.A., A.R.S, Microbiol. & Plant Path. Lab., Beltsville, MD 20705.

Phytoalexin elicitors have been hypothesized to bind to cell membrane receptors which, in turn, stimulate the production of H_2O_2. The subsequent peroxidase-mediated reduction of the H_2O_2 has been reported to result in the oxidation of certain fluorescent dyes. We have found similar results in cell-free conditioned medium from soybean suspension cultures. The introduction of oligogalacturonides to this conditioned medium stimulates the oxidation of fluorescent dyes, indicating an increase in H_2O_2. This indicates a relationship between the elicitor and certain extracellular enzyme systems which is not membrane-mediated. On the contrary, the oligogalacturonide appears to have a direct effect on one or more of the enzyme or substrate components, resulting in the bleaching of the fluorescent dyes.

A777

INDUCTION OF ACTIVE OXYGEN IN SOYBEAN CELL SUSPENSIONS BY PATHOVARS OF PSEUDOMONAS SYRINGAE. J. A. Glazener, G. L. Harmon, & C. J. Baker. U.S.D.A., A.R.S., Microbiol. & Plant Path. Lab., Beltsville, MD 20705.

Soybean (cv Mandarin) cell suspensions were treated with pectic oligosaccharides, Pseudomonas syringae pv glycinea race 6 which causes a hypersensitive response in the plant and with race 4 which is a pathogen. The role that active oxygen plays in the early stages of interaction was investigated. Active oxygen production was followed using a luminometer to measure the luminol-mediated chemiluminescence of the active oxygen species H_2O_2 and/or O_2^-(converted to H_2O_2 with superoxide dismutase). Treatment of cells with pectic oligosaccharides resulted in an immediate and transient increase in active oxygen levels. Treatments with bacteria resulted in active oxygen levels lower than untreated controls. These results sugest the presence of a H_2O_2 scavenging mechanism in bacterial treatments.

A778

GENERATION OF SUPEROXIDE ANION IN TOBACCO PROTOPLASTS IN RESPONSE TO EXTERNAL STIMULI. G.L. Harmon, J.A. Glazener, and C.J. Baker. USDA-ARS, Microbiol. & Plant Path. Lab., Beltsville, MD 20705.

Protoplasts isolated from tobacco suspension cells were treated with pectic fragment, NADPH, Pseudomonas syringae pv. syringae which causes a hypersensitive response (HR) in tobacco and a non-HR-causing mutant, B7. O_2^- was detected by monitoring the oxidation of epinephrine at pH 7.86. The highest levels of O_2^- production were seen in protoplasts treated with NADPH or pectic fragment, while levels in protoplasts treated with bacteria were comparable to untreated controls. Exogenous NADPH was not required to stimulate superoxide dismutase-inhibitable oxidation of epinephrine in tobacco protoplasts. These results suggest that O_2^- production and regulation in plant cells may occur by several different mechanisms involving various plasma membrane and/or cell wall components.

A779

PHYTOALEXIN ACCUMULATION IN ARABIDOPSIS THALIANA LEAVES INOCULATED WITH PSEUDOMONAS SYRINGAE PV. SYRINGAE. J. Tsuji[1], R. Hammerschmidt[2], and S.C. Somerville[1,2], DOE-Plant Research Laboratory[1], and Dept. of Botany & Plant Pathology[2], Michigan State University, East Lansing, Michigan 48824.

By Cladosporium TLC bioassay, antifungal activity was detected in chloroform soluble fractions of methanol extracts prepared from Arabidopsis thaliana leaves inoculated with the wheat pathogen Pseudomonas syringae pv. syringae. Little or no antifungal activity was detected in extracts prepared from leaves infiltrated with phosphate buffer, Xanthomonas campestris pv. campestris or an hrp mutant of P.s. syringae. Phytoalexin activity increased rapidly between 12 and 24 hours after inoculation with P.s. syringae and reach maximum activity between 24 and 48 hours post inoculation. Phytoalexin activity was also induced by $AgNO_3$ or $ZnCl_2$ treatment. Because Arabidopsis thaliana is amendable to many genetic and molecular techniques including T-DNA insertional mutagenesis and chromosome walking using a YAC library, this crucifer may prove to be a useful model host for studying the role of phytoalexins in disease resistance.

A780

FACTORS INFLUENCING THE PRODUCTION OF PECTINOLYTIC ENZYMES BY PSEUDOMONAS SOLANACEARUM. Jerzy Lewosz, Caitilyn Allen and Luis Sequeira. Department of Plant Pathology, University of Wisconsin, Madison 53706 U.S.A.

The production of extracellular endopolygalacturonase (PG, pI 9) in defined medium by P. solanacearum was increased by adding low MW factors from plant tissues. The effect of nutritional and other components in plant extracts on induction of PG was examined. Bacteria grown on rich media produced only small amounts of PG (pI 9). However on minimal media a significant increase in (pI 8) PG activity was observed at late logarithmic phase. PG activity was further stimulated by the adding 0.1% d-galacturonic acid (GA). Chloroform soluble compounds isolated from infected tobacco leaves significantly increased induction by GA. In addition low MW compounds released by suspension-cultured tobacco cells inhibited the production of pectinmethylesterase (PME) by the bacteria.

A781 Withdrawn

A782

INDUCTION OF LOCAL RESISTANCE TO FUSARIUM DRY ROT DISEASE IN POTATO TUBER DISCS BY NON-PATHOGENIC FUNGUS CLADOSPORIUM CUCUMERINUM. Y. Zeng and R. Hammerschmidt. Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

The induction of local resistance of potato tuber tissue to Fusarium sambucinum by Cladosporium cucumerinum was studied. Resistance to infection by Fusarium was evident within 48 hr of inoculation with C. cucumerinum. Previous work had suggested that lignin synthesis was part of the resistance of potato to C. cucumerinum, it thus could play a role in the induced resistance response. Tissue inoculated with C. cucumerinum developed levels of PAL that were, 24 hr after inoculation, 5 fold greater than the wounded controls. Peroxidase, especially acidic isozymes, were induced more rapidly by C. cucumerinum than by wounding. Lignin deposition, as reported earlier, was induced within 12 hr. C. cucumerinum, however, did not stimulate CAD activity or accumulation of chlorogenic acid over control levels. Examination of PAL gene expression in response to wounding and Cladosporium inoculation is in progress.

A784

SYNTHESIS OF INDOLE-3-ACETIC ACID BY USTILAGO MAYDIS. D. A. Navarre and K.E. Damann. Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, L.S.U. Agricultural Center, Baton Rouge, La. 70803-1720.

Corn infected with Ustilago maydis often forms galls. Bioassays were used to screen for U. maydis phytohormone production, and compounds with cytokinin or auxin activity were detected. XAD-7 chromatography and silica gel TLC was used to purify indole-3-acetic acid (IAA) from supernatants of U. maydis cultures supplemented with tryptophan. The mass spectrum of putative IAA from U. maydis corresponded to that of authentic IAA. IAA synthesis by U. maydis was monitored over time using a modified Salkowski reagent. IAA began accumulating in early log phase and peaked in the stationary phase. U. maydis cells permeabilized with Triton X-100 and fed different precursors converted indole-3-pyruvic (IPyA) acid to IAA, but not tryptamine or indole-3-acetamide. Southern analysis of U. maydis DNA did not reveal detectable homology with auxin probes (iaaM and iaaH) from Pseudomonas savastanoi. Auxin synthesis by permeabilized cells was stimulated by the addition of α-ketoglutarate and pyridoxal-5-phosphate. We suggest that in U. maydis IAA synthesis proceeds from tryptophan through IPyA and indole-3-acetaldehyde.

A785

COLD ACCLIMATION AND RESPIRATION IN MILDEWED WINTER BARLEY. M.R.McAinsh, P.G. Ayres, N.D. Paul and A.M. Hetherington. Division of Biological Sciences, University of Lancaster, Lancaster LA1 4YQ, U.K.

Winter barley was grown in cold acclimating conditions, 9^0C (8h day)/4^0C (night), and infected with a compatible race of powdery mildew (Erysiphe graminis f.sp. hordei). Controls were grown at 20^0C (16h day). Treatment effects were similar whether oxygen uptake was measured on whole leaves or isolated mitochondria. Infection stimulated total respiration in cold grown plants, with a high healthy respiration rate, and controls, with a lower rate. Whereas in healthy cold-grown plants the cyanide-insensitive component of total respiration increased notably as temperature increased, in comparable mildewed plants it remained constant, cyanide-sensitive respiration being the dominant component. The altered respiratory pattern in leaves was related to carbohydrate availability, but changes in mitochondrial activity in the presence of exogenous substrate suggest that functional efficiency was also impaired.

A786

A ROLE FOR CUTINASE IN THE EXPRESSION OF TISSUE SPECIFICITY BY FUNGAL PATHOGENS. Frances Trail and Wolfram Köller, Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456.

Previous studies indicated a role for cutinase in the expression of tissue specificity by directly penetrating fungal pathogens. Leaf-specific pathogens had cutinases with pH optima distinct from those of stem pathogens, whereas pathogens that infected both stems and leaves secreted both cutinase types. The presence of different types of cutinase secreted by these pathogens was substantiated by treatment of the culture filtrates with [^{3}H] diisopropyl fluorophosphate, followed by electrophoresis of the labelled proteins. A bioassay has been developed to explore the role of these two cutinases in penetration by a stem-specific isolate of *Rhizoctonia solani* that is nonpathogenic on leaves. Inoculum amended with cutinase from a leaf-specific pathogen penetrated the bean leaf cuticle and produced disease symptoms, whereas inoculum amended with cutinase from a stem-base pathogen remained nonpathogenic. The ultrastructure of colonization of these leaves was investigated by scanning electron microscopy. These results are the first evidence for a role of cutinase in the expression of tissue specificity by fungal pathogens.

A787

A TECHNIQUE FOR DETECTING CHITINASE, β-1,3-GLUCANASE AND PROTEIN PATTERNS ON POLYACRYLAMIDE ELECTROPHORESIS OR ISOELECTROFOCUSING GELS. S. Q. Pan, X. S. Ye and J. Kuć, Department of Plant Pathology, University of Kentucky, Lexington, Ky 40546.

A procedure is described to assay chitinase and β-1,3-glucanase isozymes and protein patterns on polyacrylamide electrophoresis (PAGE) or isoelectrofocusing gels. After electrophoresis or isoelectrofocusing, an overlay gel containing glycol chitin as substrate for chitinase was incubated in close contact with the resolving gel. Chitinase isozymes were revealed by UV illumination after staining the overlay gel with fluorescent brightener 28. The assay appeared quantitative on PAGE gels. After the resolving gel was incubated with laminarin, β-1,3-glucanase isozymes were detected with 2,3,5-triphenyltetrazolium chloride and could be quantified on PAGE gels. The resolving gel with β-1,3-glucanase bands was stained with Coomassie blue to reveal protein patterns. If both resolving and overlay gels are properly marked, chitinase and β-1,3-glucanase can be identified on gels stained with Coomassie blue.

A788

MANIPULATION OF HOST SUSCEPTIBILITY TO GRAY MOLD BY CALCIUM NUTRITION AND INHIBITORS OF ETHYLENE PRODUCTION OR ACTIVITY. Y. Elad, Dept. Plant Pathology, The Volcani Center, Bet-Dagan 50250, Israel.

Supplemental fertilization with $Ca(NO_3)_2$ (1-3mM) of tomatoes, cucumbers and roses in greenhouses reduced incidence and severity of gray mold (Botrytis cinerea). Calcium suppressed disease development in cut flowers of rose incubated at 10C more than those incubated at 4C or 20C. Leaves from calcium-treated plants exuded less nutrients than from nontreated plants. Calcium inhibited the activity of pectolytic enzymes of B. cinerea and the production of ethylene by plants. Ethylene increased susceptibility of these and other plants to gray mold. Disease was controlled by inhibitors of ethylene production or activity (AVG, AOA, polyamines, Co+2, norbornadiene, benzylaminopurine, Ag+), by an inhibitor of polyamines synthesis (DFMO) and by radical scavengers. All these compounds may have other indirect effects.

A789

THE STIMULATORY EFFECT OF AMINO ACIDS ON OOSPORE GERMINATION OF *PHYTOPHTHORA CACTORUM* IN VITRO. J. Jiang and D. C. Erwin, Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521

Mature oospores of *Phytophthora cactorum* (30 days old) were induced to become dormant by incubation in distilled H_2O at 2°C for 20 days. Germination of dormant oospores was increased significantly by 22 amino acids compared to glass distilled H_2O and the buffers used in the amino acid solutions. Germination % varied with amino acid, but all germinated within the range of 35-70% at a concentration of 10.0 mM. The minimum concentration of alanine and glycine which stimulated oospore germination was 0.1 mM. Several sugars, organic acids and salts did not stimulate oospore germination at 10.0 mM. When 1.0 mM alanine was mixed with 1.0 mM sucrose or mineral salts, the germination % was higher than with alanine alone. When oospores were incubated in 10.0 mM sucrose solution for 4 days, no germination occurred, but when the sucrose was replaced by alanine (10.0 mM) on day 4, about 40% germinated by the following day. Germination of dormant oospores was increased significantly also by alfalfa root exudate, soil extract, and oospore exudate.

A790

ANTIFUNGAL ACTIVITY FROM THE GRAINS OF CORN, SORGHUM AND WHEAT. Darnetty[1], J. F. Leslie[1], and S. Muthukrishnan[2]. [1]Dept. of Plant Pathology, and [2]Dept. of Biochemistry, Kansas State University, Manhattan, Kansas 66506.

Crude protein extracts were made from grains of 12 cultivars each of corn, sorghum, and wheat. These preparations were fractionated on 10% SDS polyacrylamide gels, western blotted and bands corresponding to chitinase and ß-glucanase identified. In sorghum, a major chitinase band (app. 29 kD) and at least two minor bands (25-28 kd) were seen. In corn and wheat at least, two major chitinase bands were observed. For ß-glucanase from corn seed, at least three bands were identified per cultivar and there was extensive polymorphism for band size. Isolates from 43 fungal species were grown on carrot juice agar and tested for their sensitivity to these protein preparations. Of the 43 species tested, isolates from 21, including Oomycetes, Ascomycetes and Deuteromycetes, had inhibited growth. *Alternaria alternata*, *Trichoderma viride*, and binucleate *Rhizoctonia* from AG-A, AG-G, and AG-I were particularly strongly inhibited.

A792

DRRG49 AND DRRG206 GENES ARE STRATEGICALLY ACTIVE IN PEA SEEDLINGS RESISTANT TO RACES OF *FUSARIUM OXYSPORUM* F. SP. *PISI*.
L. A. Hadwiger, C. Chiang, and D. Horovitz, Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

Pea lines M410 (Sus) and Vantage (Res) have common genetic backgrounds except for the single-factor resistance to Race 1 of *F. oxysporum* f. sp. *pisi*. We acquired DRRG49 and 206 from a cDNA library of Alaska pea pods expressing non-host resistance to *F. solani* f. sp. *phaseoli*. These genes are expressed as pea seedlings develop in Race 1-infested soil, but the mRNA accumulates most intensively in Vantage (Res) a few days prior to the corresponding appearance of severe wilt symptoms in M410 (Sus). These results indicate the "master" single Mendelian resistance traits may potentiate multiple "slave-like" gene responses to resist multiple pathogen challenges. The combined activity of DRRGs, such as gene 49 which codes for a major portion of the inducible pea proteins and more recently has been found in many other plants, may provide major functions in resistance generated against multiple pathogens.

A793

THE DISEASE RESISTANCE RESPONSE GENE 49 PRODUCT ACCUMULATES IN NUCLEI, CELL WALLS, AND VASCULAR SYSTEMS OF PEA PODS. B. Allaire, C. Chiang, and L. A. Hadwiger, Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

Messenger RNA from a PR-like gene, DRRG49-c, accumulates in pea pod tissue following the challenge of both fungal and bacterial pathogens or after treatment with biotic and abiotic elicitors. Its predicted 17 Kd product accumulation has been followed in pea tissue with Western blots utilizing antisera developed against a DRRG49-c/β-galactosidase fusion antigen. Light and electron microscopic immunoassays indicate the 17 Kd protein accumulates in the nuclei and walls of endocarp cells proximal to the added inoculum of *Fusarium solani* f. sp. *phaseoli* (incompatible reaction) within 2-6 hours. Also, copious quantities appear in vascular tissue. The regional concentration and potential movement in vascular tissue suggests this protein protects or signals nuclei in cells not immediately in contact with the fungal spore.

A794

SCREENING FOR GENES INDUCED IN A GRASS/FUNGUS SYMBIOSIS.
H.-F. Tsai, C. L. Schardl, and M. R. Siegel. University of Kentucky, Lexington KY 40546-0091.

Acremonium coenophialum is a mutualistic, seed-disseminated endophyte of tall fescue (Festuca arundinacea), an important pasture, forage and turf grass. We wish to identify cDNA clones of plant and fungal genes that are modulated in symbiosis. Poly(A)+RNA was isolated from cultured endophyte, uninfected grass, and the grass/fungus symbiote. The grass and symbiote mRNAs were from the meristems and surrounding leaf sheaths, where the endophyte is normally concentrated. The cDNAs were cloned in λUni-ZAP XR (Stratagene), a hybrid phage/phagemid vector. The numbers of primary plaques in each library were: 4×10^5 from the endophyte, 1.4×10^6 from the grass, and 2×10^6 from the symbiote. The fungus and plant libraries are being used as control competitors in a subtractive-hybridization screening to identify clones of mRNA species whose expression is enhanced in the symbiotic interaction.

A795

ISOLATION OF A DNA FRAGMENT FROM *ASPERGILLUS NIDULANS* CONTAINING A GENE FOR RESISTANCE TO THE ANTI-FUNGAL COMPOUND LY214352.
G. E. Davis, C. Waldron, and G. D. Gustafson, DowElanco, P. O. Box 708, Greenfield, IN 46140.

DNA from a chemically-induced *Aspergillus nidulans* mutant (52-6) resistant to the anti-fungal compound LY214352 was cloned in the cosmid vector pKBY2. This cosmid library was screened until a single cosmid (6A6) which could transform an LY214352-sensitive strain of *A. nidulans* to LY214352-resistance was isolated. The DNA insert in cosmid 6A6 is approximately 35,000 base pairs (bp) and subclones of that insert containing a 7250 bp Sal I fragment or a 5350 bp Hind III fragment (nested within the Sal I fragment) will transform LY214352-sensitive strains of *A. nidulans* to LY214352-resistance. Subclones of these Sal I and Hind III fragments are now being tested in our transformation system in order to pinpoint the location of the gene responsible for the LY214352- resistance trait. A cosmid library prepared using DNA from a wild-type (LY214352-sensitive) strain of *A. nidulans* has been used to isolate a DNA fragment containing the LY214352-sensitive form of this gene.

A796

MUTANTS OF *COCHLIOBOLUS HETEROSTROPHUS* WITH ALTERED ABILITIES TO SECRETE CELL WALL DEGRADING ENZYMES. L. K. Lyngholm, C. A. Spike, and C. R. Bronson, Iowa State University, Ames, Iowa 50011.

The goal of this research is to determine the role of cell wall degrading enzymes in the pathogenicity of *C. heterostrophus*. An efficient mutagenesis protocol has been developed based on UV irradiation of protoplasts. The survivors are being screened for more, less, or no secretion of 4 enzymes relative to wildtype. To date, 7 protease, 5 β-xylosidase, 3 polygalacturonase, and 2 xylanase mutants have been tentatively identified. Segregational analysis has confirmed the genetic control of the mutant phenotypes of 2 low and 4 non-producers of protease and 1 high producer of β-xylosidase. The remaining putative mutants are being tested. Six of the protease mutants have been inoculated onto maize and found to remain pathogenic. After backcrossing, the pathogenicity of the mutants relative to wildtype will be measured quantitatively by determining infection efficiency and lesion size on maize.

A798

GENETICS OF RUST RESISTANCE IN PHASEOLUS VULGARIS PLANT INTRODUCTION 181996. J. R. Stavely, Microbiology and Plant Pathology Laboratory, ARS, USDA, Beltsville, MD 20705.

Phaseolus vulgaris plant introduction (PI) 181996 is resistant to all 33 races of the bean rust fungus, Uromyces appendiculatus, that have been identified at Beltsville since 1983. Crosses were made between certain snap and pinto bean cultivars and PI 181996. Each plant in the F_1, F_2, and some F_3 populations was inoculated with eight selected races of U. appendiculatus and rust reactions were recorded 14-15 days later. The F_2 populations from each cross segregated in ratios that indicated that PI 181996 contains one or two dominant resistance genes per race. Segregation in these F_2 populations and those from backcrosses with susceptible cultivars indicated that PI 181996 contains a complex locus of tightly linked genes that is usually inherited as a unit conferring resistance to all races. The Up-2 gene of bush snap beans that is effective against 14 races and the resistance genes of pinto cv. 'Olathe' for 23 races are distinct from the resistance genes in PI 181996.

A799

SEGREGATION FOR DOMINANT VIRULENCE IN THE BEAN RUST FUNGUS. J.W. McCain, J.V. Groth, and A.P. Roelfs, Dept. of Plant Pathology and USDA/ARS Cereal Rust Laboratory, The University of Minnesota, St. Paul, MN 55108.

Segregation for virulence/avirulence was studied in Uromyces appendiculatus for 33 single-uredial isolates representing nine field collections. Isolates were induced to form telia and thence pycnia for mass-self-fertilization on a compatible bean (Phaseolus vulgaris) cultivar. The parental uredospores and the mass-selfed-progeny were compared on eight differential bean lines. Progeny segregated in 86 of 237 host/parental isolate combinations (36%). Of 116 avirulent line/isolate combinations, 50 segregated, suggesting recessive virulence in the mass-selfed progeny. However, 36 of 121 virulent line/isolate combinations also segregated after mass-selfing, suggesting dominant virulence (studies are not complete to determine how many actual virulence genes are segregating in these rust collections). All nine collections were polymorphic for virulence on from five to eight of the bean lines, and all had members that segregated for dominant virulence on from two to six of the lines.

A800

INTER- AND INTRA- SPECIES HYBRIDISATIONS BETWEEN PATHOGENIC FUSARIUM SP. BY PROTOPLAST FUSIONS AND HYPHAL ANASTOMOSES. C. Madhosingh, Agriculture Canada, London, Canada, N6G 2V4.

Pathogenic isolates of Fusarium graminearum Schwabe (FG), F. oxysporum f.sp. lycopersici (Sacc.) Snyder & Hansen (FOL) and f.sp. radicis lycopersici Jarvis & Shoemaker (FORL), treated with the mutagen nitrosoguanidine, produced a number of cycloheximide (C) and mycostatin (M) resistant mutants. Protoplast fusions and hyphal anastomoses were promoted between FOL (C), FORL(M) and FG(C) in liquid and agar cultures. Samples from these cultures were inoculated on medium containing 'resistant levels' of both antibiotics. Isolates growing on the double antibiotic medium, were considered hybrids. Hyphal tip isolations from the hybrids were maintained on double antibiotic slants. The hybrids demonstrated differences from the parents in their protein and enzyme patterns, pathogenicity, growth and morphology.

A801

INHERITANCE OF STRIPE RUST RESISTANCE IN EIGHT WHEAT CULTIVARS POSTULATED TO HAVE RESISTANCE GENES AT *Yr3* AND *Yr4* LOCI. Xianming Chen and Roland F. Line. USDA/ARS, Dept. of Plant Pathology, Washington State Univ., Pullman, WA 99164.

Cappelle Desprez (CD), Druchamp (DRU), Hybrid 46 (H46), Minister (MIN), Nord Desprez (ND), Stephens (STE), Vilmorin 23 (V23), and Yamhill (YAM) have been postulated to have resistance genes at the *Yr3* locus and/or *Yr4* locus. Seedlings of parents and F1, F2, and BC1 progeny from reciprocal, diallel crosses among the cultivars and of the eight cultivars with Chinese 166 (*Yr1*) were tested for resistance to selected North American races of *Puccinia striiformis*. YAM has three resistance genes. Each remaining cultivar has two genes. No common loci were detected for crosses of H46 with DRU, MIN, or STE and of YAM with STE. MIN, CD, DRU, ND, and STE have genes at the *Yr3* locus, and the gene in MIN is different from the gene in the other cultivars. H46, V23, and YAM have genes at the *Yr4* locus, and the gene in H46 is different from the gene in V23 and YAM. The second gene in H46 is not at the *Yr3* locus, as previously reported.

A802

ADAPTING A SINGLE-LOCUS, OVERDOMINANCE SELECTION MODEL TO THE AUTOECIOUS, MACROCYCLIC LIFE CYCLE OF Uromyces appendiculatus. D. C. Linde and J. V. Groth, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Sexual populations of the bean rust fungus are frequently characterized by high percentages of isolates heterozygous for "unnecessary" virulence genes. Overdominance is one possible explanation for the high frequency of virulence heterozygotes. The single locus, overdominance selection model of classical population genetics was adapted to the autoecious, macrocyclic life cycle of the bean rust fungus, and used to investigate the influence of the size of virulence and avirulence homozygote selection coefficients and number of repeating cycles per year on the change in frequencies of virulence heterozygotes both within and between years for 40 years. A Monte Carlo simulation was made to investigate the effect of varying numbers of repeating cycles per year on the frequency of heterozygotes. Proof of overdominance may be obtained by sampling early and late in severe epidemics and observing an increase in heterozygote frequency for several virulence genes.

A803

IS801, AN UNUSUAL TRANSPOSABLE ELEMENT OF *PSEUDOMONAS SYRINGAE* PATHOVAR *PHASEOLICOLA*. M. Romantschuk*, G. Richter, and D. Mills, *Department of General Microbiology, University of Helsinki, SF-00100, Helsinki, Finland, and Department of Botany and Plant Pathology and Genetics Program, Oregon State University, Corvallis, Oregon, 97331-2902.

A transposable element, designated IS801, has been isolated from strain LR700 of *P. syringae* pv. *phaseolicola*, a pathogen of bean. Partial and complete copies of the element reside on a cryptic plasmid of LR700, and in the genomes of other *P. syringae* pathovars. Two copies of IS801 that had transposed into different sites of an entrapment plasmid, pUCD800, as well as a third copy which has not yet been observed to transpose, have been cloned. IS801 generates a duplication of a five base pair (bp) target site. The element is 1517 bp in length, contains two open reading frames of 1230 bp and 597 bp on opposite strands, and is unusual in that it contains no direct or inverted repeats at its termini.

A804

NITRATE NONUTILIZING MUTANTS OF COLLETOTRICHUM. Brooker, N. L., Leslie[1], J.F., and Dickman, M. B. Departments of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722 and [1]Kansas State University, Manhattan, KS, 66506.

Colletotrichum spp. represent a diverse and complex group of economically important fungal pathogens. Methodologies used for analyzing relatedness of species and subspecies have been complex, and in many cases contradictory. An alternative approach is based on vegetative compatibility. Mutants unable to reduce to NO_3 to NH_4 (nit mutants) arose spontaneously as sectors resistant to $KClO_3$ in strains from selected isolates. Nit mutants could be divided into four phenotypic classes. These classes presumably represent mutations at a nitrate reductase structural locus (nit1), global nitrogen regulatory locus (nnu) a nitrate assimilation pathway-specific regulatory locus (nit3), and several loci that affect the assembly of a molybdenum-containing cofactor (Nit M). Frequencies of nit mutations, mutant morphologies, physiological complementations of the nit mutants, and preliminary vegetative compatibility studies suggest that the isolates examined are all genetically distinct.

A805

VEGETATIVE COMPATIBILITY AMONG ISOLATES OF COLLETOTRICHUM GLOEOSPORIOIDES F. SP. AESCHYNOMENE. **R. J. Chacko, J. C. Correll, G. J. Weidemann and D. O. TeBeest. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.**

Field isolates of Colletotrichum gloeosporioides f. sp. aeschynomene (CGA) from Northern Jointvetch and Indian Jointvetch in Arkansas and Louisiana were examined for vegetative compatibility. Nitrate non-utilizing (nit) mutants were generated from isolates using minimal medium amended with potassium chlorate. Auxotrophic mutants and nit mutants derived from auxotrophic mutants also were generated from several isolates and paired to study heterokaryosis. Pairings of phenotypically distinct Nit1 and NitM mutants as well as several auxotrophic mutants suggest that all isolates are vegetatively compatible. Mycelial transfers of wild type isolates grown on water agar plates were examined microscopically and hyphal fusions were observed in all strains. Analysis of mycelial blocks taken from heterokaryotic colonies suggests localization of the heterokaryotic region within the central portion of the colony. Results indicate that CGA isolates from different geographic locations are vegetatively compatible.

A807

POPULATION DYNAMICS OF STRAINS OF XANTHOMONAS CAMPESTRIS DIFFERING IN AGGRESSIVENESS ON CITRUS. D.S. Egel, J.H. Graham, and T.D. Riley. University of Florida, Citrus Research and Education Center, Lake Alfred, FL 33850.

Strains of Xanthomonas campestris cause bacterial canker (X. c. citri) and bacterial spot (X. c. citrumelo) of citrus, respectively. Strains of both pathovars were compared for their capability to grow endophytically and epiphytically. These capabilties were postively correlated with aggressiveness. Endophytic populations were indicative of epiphytic populations, and might be used to predict field spread of a particular aggressiveness type. Strain X cultivar interactions occurred when aggressive strains of X. c. citrumelo were compared on cultivars Swingle citrumelo and Duncan grapefruit. X. c. citri and the aggressive strain of X. c. citrumelo on Swingle citrumelo were the only strains capable of significant growth in citrus leaves.

A808

CHARACTERIZATION OF XANTHOMONADS FROM ARACEAE BY FATTY ACID ANALYSES. N. C. Hodge, A. R. Chase, and R. E. Stall. Plant Pathology Department, University of Florida, Gainesville, FL 32611.

Based on analyses of fatty acid profiles of xanthomonads from different genera of Araceae 150 strains were divided into subgroups. Strains from Aglaonema, Anthurium, Colocasia, Dieffenbachia, Epipremnum, Philodendron, and Syngonium all had similar ratios (2:1) of the predominant acids 15:0 iso and 15:0 anteiso, respectively. Subgroups were based on quantitative differences among other unsaturated and hydroxy fatty acids. None of the subgroups was consistently associated with strains from a particular host. The profiles of 12 strains from Xanthosoma differed from the strains isolated from other aroids by the unique 1:2 ratio of the 15:0 iso to 15:0 anteiso fatty acids. The strains from Xanthosoma were pathogenic to other aroids.

A810

ISOLATION OF EXTRACELLULAR POLYSACCHARIDES PRODUCED BY *Clavibacter michiganense* subsp. *sepedonicus*. A. Westra and S. A. Slack, Department of Plant Pathology, Cornell University, Ithaca New York 14853.

Fluidal strains of *Clavibacter michiganense* subsp. *sepedonicus*, (Cms) the causal agent of bacterial ring rot of potato, were found to produce, *in vitro*, four extracellular polysaccharide components that could be separated on the basis of their size and charge. Components I and II were large (>2 x 10^7 and 4.5 x 10^6 daltons, respectively), acidic polysaccharides that appear to be aggregates of a smaller (20,000 dalton) polysaccharide, component III. Components I, II, and III were of similar neutral sugar composition (1 fucose: 5 mannose: 1 galactose: 1 glucose) and reacted similarly with polyclonal antisera specific for whole Cms cells. Components I and II, when treated with 0.1% SDS, dissociated into a subunit of a size and composition similar to that of component III. A fourth component, IV, differed considerably from the other three components in that it was neutral, composed primarily of mannose, and did not react with polyclonal antisera specific for whole Cms cells. All components were found to be homogenous based on ion exchange or gel permeation chromatography, compositional analysis, and reaction with polyclonal antisera in Ouchterlony agar double diffusion serology tests. No evidence for the presence of glycopeptides was found.

A811

THE ORIGIN OF CONJUGAL TRANSFER OF THE *Agrobacterium tumefaciens* TI PLASMID pTiC58. D. M. Cook and S. K. Farrand, Department of Plant Pathology, University of Illinois at Urbana-Champaign, IL 61801.

Ti plasmids of *Agrobacterium tumefaciens* are conjugal plasmids and their transfer is induced by opines secreted from crown galls. In well characterized conjugal plasmid systems such as F, the process of conjugation initiates at a cis-acting site known as the origin of conjugal transfer or oriT. We have localized an oriT on the *A. tumefaciens* plasmid pTiC58 to a region encoding conjugal transfer loci TraI and TrbI, and also acc (the catabolic locus for the conjugal opines, agrocinopines A and B). A cosmid clone of pTiC58, pTHB58, was mobilized by a transfer-constitutive mutant, pTiC58Tra^c, at a frequency of 2.7 X 10^{-2} per input donor. A 0.7 kb oriT-active fragment was subcloned which is mobilized to recipients at a frequency of 5.4 X 10^{-4} per input donor. This fragment maps to the TraI region of the Ti plasmid. We have sequenced this fragment and have identified several inverted repeats which may act as the nic site at which a single-stranded cleavage occurs allowing subsequent DNA transfer. We also demonstrated that pTiC58-derived oriT clones can be mobilized by another nopaline-type Ti plasmid, pTiT37, and by a transfer-constitutive octopine-type Ti plasmid pTi15955.

A812

TRANSFORMATION OF A XYLEM-LIMITED BACTERIA (*CLAVIBACTER XYLI* SUBSP. *CYNODONTIS*) OF BERMUDA GRASS TO EXPRESS INSECTICIDAL *B. THURINGIENSIS* TOXIN. M.C. Metzler, T.A. Chen. Martin Hall, Cook College, Rutgers University, New Brunswick, N.J. 08903.

Clavibacter xyli subsp. *cynodontis* is a gram-positive coryneform bacteria which lives non-pathogenically in the xylem of bermuda grass. Our goal is to transform the bacteria with a gene coding for one of the insecticidal proteins from *Bacillus thuringiensis*. Such a bacteria could then be transferred back into the host and would presumably confer upon it insect resistance. We have developed a procedure for transforming the bacteria with a plasmid using electroporation, and are currently cloning a *B.t.* toxin gene into the plasmid in preparation for transforming it into the bacteria. The transformed bacteria will then be transferred into uninfected bermuda grass and the grass tested for insecticidal properties. In addition, we are infecting various other turf grasses with the bacteria to determine if it can grow in other hosts.

A813

PHENOTYPIC PLASTICITY AFFECTING EPIPHYTIC SURVIVAL IN PSEUDOMONAS SYRINGAE. M. Wilson and S. E. Lindow, Department of Plant Pathology, University of California, Berkeley, CA 94720.

P. syringae cells cultured in either solid or liquid media, or harvested from inoculated bean plants, were sprayed onto greenhouse-grown or field-grown bean plants and then incubated, either in a growth chamber at low relative humidity and high light intensity, or in the field. Recoverable P. syringae populations decreased 10-500 fold in the first 12 h, then increased again in the following 48 h. In the growth chamber, the population of liquid-cultured cells decreased on average 260-fold, compared to the plant-harvested and solid-cultured cells which decreased on average only 90-fold and 50-fold, respectively. In the field, similar patterns of survival were observed, but in addition the plant-harvested cells started to multiply earlier and reached a higher final population than other cell types. These results suggest that studies of bacterial epidemiology using cells cultured in vitro will not be accurate predictors of field behavior.

A815

DEVELOPMENT OF AN INDEXING SYSTEM FOR CONTROL OF BACTERIAL BLIGHT OF ANTHURIUM. D. Norman, A. Alvarez, and A. Benedict. University of Hawaii, Honolulu, HI 96822.

Production of disease-free plants and the development of an indexing system to detect latent infections in symptomless plants is a first step in controlling anthurium blight, a widespread disease that has caused significant losses to the anthurium industry. A diagnostic system for symptomless plants potentially infected by Xanthomonas campestris pv. dieffenbachiae (Xcd) was developed. Indexing involved assays of tissue

sections using selective media followed by confirmation with 2 monoclonal antibodies (mAbs). In addition a panel of 9 mAbs was utilized to serologically differentiate strains and monitor the spread of Xcd. One thousand symptomless top cuttings were removed for propagation and simultaneously assayed for Xcd. Of the symptomless plants 0.4% were found to be infected with Xcd and were removed. Plants were kept in blocks separated from commercial plantings with plastic and saran cloth barriers. To monitor possible spread of Xcd back into the mother block 245 Xcd strains from symptomatic plants in the production area were isolated and serotyped. Indexing has indicated where changes are necessary to develop a clean mother block system.

A816

INHIBITION OF VEGETATIVE GROWTH OF *Armillaria ostoyae* AND *A. bulbosa* BY RED PINE MYCORRHIZOPLANE STREPTOMYCETES. D.M. Becker, S.M. Paetschow, S.T. Bagley, and J.N. Bruhn. Michigan Technological University, Houghton, MI 49931

Studies are being initiated to determine if streptomycetes can have inhibitory effects on the white rot wood decay fungi *Armillaria ostoyae* (NABS I) or *A. bulbosa* (NABS VII). The five streptomycete morphotypes being tested were originally isolated from the mycorrhizoplane of healthy red pine seedlings growing in four year old plantations which have experienced approximately 12% mortality due to root rot caused by *A. ostoyae*. However, *A. bulbosa* is also abundant in the same plantations. The streptomycetes selected have demonstrated inhibitory effects *in vitro* on growth of *Laccaria* and *Thelephora* spp. In these studies, an *Armillaria* isolate is inoculated onto MMN agar previously inoculated with a single streptomycete morphotype. Radial mycelial growth away from the streptomycete culture was measured. Three clones representing each *Armillaria* sp. have been tested. Compared to controls, inhibition ranged from 10-100%, depending on the streptomycete morphotype used. The effects of these streptomycetes on rhizomorph growth are also under study. Further work may involve characterization of the inhibitory compounds.

A817

CALCIUM CHLORIDE ALLEVIATION OF SALT STRESS IN RHIZOBIUM LEGUMINOSARUM BIOVAR VICIAE. C. Chien, R. Rupp, and C. S. Orser, Department of Bacteriology and Biochemistry, University of Idaho, Moscow, Idaho 83843.

R. leguminosarum biovar viciae strain C1204b exhibits a dramatically reduced growth rate when exposed to 200 mM NaCl. Common osmolytes, such as proline, glycine betaine, choline, glutamte and trehalose, do not relieve the sodium toxicity. However, the addition of calcium chloride relieves the inhibition of growth caused by salt stress to the free-living microsymbiont. The growth rate of C1204b in the presence of NaCl steadily increased with increasing $CaCl_2$ concentration from 1 to 6 mM. Moreover, other divalent cations ($StCl_2$, $MgCl_2$, $BaCl_2$) are also able to ameliorate salt toxicity, although to a lesser degree than $CaCl_2$. Tn5-induced mutants were generated which no longer respond to $CaCl_2$ alleviation of NaCl toxicity. The mutants also do not respond to other divalent cations. Complementing cosmid clones have been isolated for the $CaCl_2$ mutants from a genomic bank of C1204b.

A819

A COMPARISON OF METHODS FOR ASSESSING FUNGISTATIC CAPACITY OF SOILS. T.L. Wacker, L.B. Kao, and J.L. Lockwood. Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

The relative sensitivities of conidia of three fungi, Cochliobolus sativus, C. victoriae, and Fusarium graminearum, to fungistasis on three different soils, were compared using two assay methods. One method involved the addition to soil of a series of glucose-peptone concentrations. The other involved dilution of the soil with different concentrations of sand. Conidia placed on membrane filters were incubated on the soils overnight in closed Petri dishes at 23°C, then stained with phenolic rose bengal and germination percentage determined. The order of sensitivity to fungistasis of the three fungi was the same for two of the soils: C. sativus >, C. victoriae >, F. graminearum. In the third soil C. sativus was least sensitive. Though the order of sensitivity of the fungi differed between soils, the two assay methods gave the same order of sensitivity on all three soils.

A820

PROFESSIONALISM IN PLANT PATHOLOGY - CAN IT BE TAUGHT? C. J. D'Arcy and W. L. Pedersen, Department of Plant Pathology, University of Illinois, 1102 S. Goodwin Ave., Urbana, IL, 61801.

Professionalism has several aspects, which include the methods, character and standards of individuals in the field. Since we believe that some aspects of professionalism can be taught, in 1982 we initiated a course for graduate students on professionalism in plant pathology. The objectives of the course are to convey practical information about our field and to encourage development of individual professional attitudes. The ungraded course is totally voluntary. Many guest speakers are invited to share their expertise and experience on a wide range of topics. Some topics which have been included are scientific writing, authorship, editorial processes, manuscript reviewing, job search and applications, types of jobs, interviews, benefits, seminar presentation and evaluation, grant funding sources and review processes, and teaching methods and evaluation. Sample "scenarios" which are used to generate class discussion will be displayed.

A821

AN IMPROVED TECHNIQUE FOR CREATING AND MAINTAINING LEAF WETNESS BY USE OF ULTRASONIC HUMIDIFIERS. B. J. Steffenson and T. G. Fetch, Jr. Dept. of Plant Pathology, North Dakota State Univ., Fargo, ND 58105.

Many plant pathogens require free moisture for infection. Laboratory studies of this requirement indicate that improvements for inducing leaf wetness can be made using ultrasonic humidifiers (UHs). UHs produce a fine mist (about 5µm diameter droplet) by means of a piezoelectric transducer, and in our studies, have proven effective in creating and maintaining uniform leaf wetness on barley and wheat. Using UHs in plexiglass/stainless steel incubation chambers, we have obtained reliable and uniform infection with several biotrophic and nonbiotrophic pathogens of barley and wheat. Additionally, UHs are inexpensive, portable, and come equipped with humidistats for sustaining a specific level of humidity. These results substantiate the utility of UHs in creating environments that are conducive for infection by many pathogens.

A822

A leaf-disk inoculation technique for evaluation of sunflower downy mildew (*Plasmopara halstedii*) resistance. T. J. Gulya. USDA Northern Crop Science Laboratory, Box 5677, Fargo ND 58105.

A leaf-disk inoculation (LDI) technique was developed which permitted determination of susceptibility within 7 days, compared to 14 days for conventional seedling inoculation. Leaves were harvested in mid-morning, dusted with 400 mesh carborundum powder and rubbed gently. Leaf disks (1.5 cm diam) were cut, avoiding main veins, and were vacuum-infiltrated with distilled water. The water-soaked disks were immersed in a zoosporangia suspension (2×10^4/ml) for 3-5 hr, placed abaxial side on 7% water agar plates, and incubated in growth chambers at 15 C with 16 hr photoperiod of 150 microE/m/sec. Sporulation, predominantly around disk edges, was observed with a dissecting microscope. The carborundum-wounding and water-soaking greatly increased the number of disks with sporulation and sporulation intensity. Leaf disks from mildew-resistant genotypes exhibited sparse sporulation on 5% or less of the disks. Results from LDI corroborated well with those from conventional seedling inoculations.

A823

A DOT-IMMUNOBINDING ASSAY FOR CITRUS TRISTEZA VIRUS M.A. Rocha-Peña and R.F. Lee. University of Florida. Citrus Research and Education Center. Lake Alfred, FL 33850.

The double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) has been used extensively for the detection of citrus tristeza

virus (CTV). A dot-immunobinding assay has been developed as an alternate serological technique for CTV detection. Both polyclonal and monoclonal polyspecific antibodies were tested in a series of different solutions and incubation times. Differences were found in the reactivity of some of the antibodies tested and in the suitability of buffers used.

A824

A COMPARISON OF THREE SELECTIVE MEDIA FOR ENUMERATION OF SCLEROTIA OF *MACROPHOMINA PHASEOLINA*. G.L. Cloud and J.C. Rupe, University of Arkansas, Fayetteville 72701.

Selective media for enumeration of sclerotia of *Macrophomina phaseolina* have temporal limitations in relation to incubation periods. The selective chemical compounds used also are either highly toxic or difficult to obtain. A new selective medium (PK) consisting of 39 g/L of potato dextrose agar, 100 mg/L rifampin, and 224 mg a.i./L metalaxyl is compared against two other selective media (MP, MSK) used commonly to enumerate *M. phaseolina* sclerotia. Twenty field soils in Arkansas natural infested with *M. phaseolina* were assayed as well as sterilized soil infested with sclerotia produced on vermiculite (added on wet weight basis and then diluted serially to 1:4 w/w). PK had an overall significantly higher ($P < 0.01$) sclerotial count in natural field soil than did MP and MSK. Sclerotial counts in 7 out of 20 natural field soils were significantly higher ($P < 0.01$) on PK than MP or MSK. No significant differences in sclerotial counts among the three media were observed in the artificially infested soil. PK is a preferred medium because it is efficient in enumerating sclerotia in natural soil, contains fewer chemical compounds, and the incubation period for PK ranges from 4 to 14 days compared to no more than 4 days for MP and after 7 days for MSK.

A825

EFFECT OF ELEVATED CO_2 AND CULTURE MEDIUM ON THE SURVIVAL OF IN VITRO *RUBUS* PLANTS DURING THERMOTHERAPY. J. D. Postman, B. M. Reed, USDA/ARS National Clonal Germplasm Repository, 33447 Peoria Road, Corvallis, Oregon 97333.

Survival of raspberry plants growing in vitro in heat-sealed gas-permeable polypropylene bags was compared on multiplication and rooting media at two levels of atmospheric carbon dioxide. Following a gradual temperature increase from 30 C to 40 C over 13 days, rooted plants in a medium without growth hormones survived better than unrooted plants in multiplication medium. In several experiments using plants on rooting medium, 1.0% atmospheric CO_2 provided no apparent benefit over ambient CO_2 (0.3%). In one test, 44% of 84 plants survived 75 days at temperatures alternating every 4 hours between 30 C and 38 C. Heat-sealed polypropylene bags were practical culture containers for these in vitro studies as they prevented drying of the medium, allowed gas exchange, and eliminated the possibility of contamination by outside organisms. The therapy temperatures and length of treatments were comparable to protocols used to successfully eliminate many viruses from shoot tips of whole plants. In vitro thermotherapy can streamline the elimination of viruses from clonally propagated plant germplasm, in a smaller space and with fewer inputs than conventional thermotherapy.

A826

SURVEY OF BARLEY YELLOW DWARF VIRUSES IN CEREAL NURSERIES IN LATIN AMERICA AND ELSEWHERE DURING 1988 AND 1989. G. N. Webby; R. M. Lister, Dept. Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907; P. A. Burnett, CIMMYT, Mexico.

A survey of cereal nurseries in Latin America and elsewhere was initiated in 1988 to determine the incidence of barley yellow dwarf viruses (BYDV) in each nursery and the frequency with which specific BYDV serotypes occurred. Random and symptomatic collections of samples were tested in double antibody sandwich ELISA against a panel of polyclonal antisera to the MAV, PAV, SGV, RPV and RMV serotypes of BYDV. In Mexican nurseries, MAV, PAV, RPV and RMV serotypes were all common with RMV the most common, particularly in random collections. MAV was the most common serotype in the nurseries sampled in Ecuador and Colombia, while PAV was the most common serotype in three nurseries in Chile. MAV, PAV and SGV serotypes were all found in nurseries in Paraguay, Uruguay and Brazil. The incidence of BYDV varied between nurseries and between seasons. In tests on mostly symptomatic samples from six countries in Africa, PAV and RMV were the most common serotypes found. PAV was also the most common serotype in symptomatic samples from Pakistan.

A827

EFFECT OF SEED TREATMENT FUNGICIDES AND HERBICIDE ANTIDOTES ON SEED DETERIORATION OF SORGHUM IN SOUTH TEXAS STORAGE ENVIRONMENTS. G.N. Odvody, N.M. Spencer, and J. C. Remmers, Texas A&M Expt. Station, Corpus Christi, TX 78410.

Seed of sorghum lines and hybrids were treated with phenylamide fungicides, captan, TCMTB, and herbicide antidotes either alone or in combination and subjected to a warehouse storage environment in Corpus Christi in 1987, 1988, and 1989. Seed viability (evaluated every 3 wk from late March for 24-27 wk) of most treatments dramatically declined after 12-18 wk in response to high temperatures especially if combined with high relative humidity. In all years, decline in seed viability from greatest to least was associated with herbicide antidote/phenylamide combinations, herbicide antidotes alone, phenylamides alone, nontreated seed, and captan alone. Rates of decline were variable by year in seed receiving combination treatments that included captan. In 1987, only captan and TCMTB protected seed from invasion by *Aspergillus* and *Penicillium* spp. At experiment termination in 1988 and 1989, various fungi were isolated from dead or dying seed of all treatments but were lowest from seed of any captan treatment.

A828

DECREASED WINTER SURVIVAL OF SUBTERRANEAN CLOVER INFECTED WITH SUBTERRANEAN CLOVER RED LEAF VIRUS. M. R. McLaughlin, USDA, ARS, Crop Science Research Laboratory, Forage Research Unit, P. O. Box 5367, Mississippi State, MS 39762-5367.

Trifolium subterraneum L. plants were transplanted to the field as 6-wk-old seedlings in mid-Oct. 1989 in an experiment to test the hypothesis that virus infection predisposes plants to winterkill. Plants were set 15 cm apart in a 4 x 4 grid within microplots mulched with 75-cm-squares of DuPont TYPAR landscape fabric separated by 60-cm-wide fescue borders. Treatments consisted of two levels of virus inoculation across two cultivars in a 2 x 2 factorial experiment (RCB) with 4 replications. Inoculations were made in late Nov. with a Mississippi isolate of subterranean clover red leaf (soybean dwarf) luteovirus (Phytopathology 78:1584), using viruliferous Acyrthosiphon pisum. Freezing temperatures in late December reduced stands by more than 50%. Counts of plants surviving in mid-Feb. 1990 were subjected to ANOVA. Significantly fewer ($p=0.05\%$) inoculated than noninoculated plants survived (23% vs 44%), thus supporting the hypothesis.

A829

AN OUTBREAK OF MAIZE CHLOROTIC MOTTLE VIRUS IN HAWAII AND POSSIBLE ASSOCIATION WITH THRIPS. X. Q. Jiang, D,. R. Wilkinson, and J. A. Berry, Pioneer Hi-Bred International, Inc., 7250 NW 62nd Ave., P.O. Box 1004, Johnston, IA 50131.

Maize Chlorotic Mottle Virus (MCMV), a vital component of the CLN (corn lethal necrosis) complex, was surveyed for the first time in Hawaii using ELISA. The disease spread quickly on the island of Kauai and appeared closely related to an increasing population of thrips which could not be controlled by spraying due to a long period of rainfall. Biotin-labelled indirect ELISA confirmed that the virus was associated with the thrips. Thrip transmission studies are currently in process. Although MCMV has been reported to be non-seed transmittable, all parts of immature ears (silk, pericarp, embryo, cob, and whole seed) were shown positive to MCMV antibody. Studies are currently underway to survey other possible vectors and to test mature seeds.

A830

OCCURRENCE AND CHARACTERIZATION OF A VIRUS INFECTING YARD-LONG BEAN (VIGNA UNGUICULATA SUBSP. SESQUIPEDALIS) ON GUAM. C. A. Kimmons, G. C. Wall, and D. A. Nelson, CALS/AES, Univ. of Guam, Mangilao, GU 96923; and B. B. Reddick, Dept. of Ent. and Pl. Path., Univ. of TN, Knoxville 37901-1071.

A virus causing mosaic disease of Vigna unguiculata subsp. sesquipedalis on Guam was isolated and partially characterized. Systemically-infected bean plants develop mosaic, vein-banding, and leaf deformation. In host-range tests, 49 plant species, cultivars, or breeding lines were sap-inoculated. Thirty-nine developed viral symptoms or were shown to be infected by return inoculations on Chenopodium amaranticolor, a local-lesion host. The virus was transmitted in a non-persistent manner by the aphid Aphis craccivora. In Protein-A sandwich enzyme-linked immunosorbent assay (P-AS ELISA), sap from virus-infected plants reacted positively with antiserum for strain W of blackeye cowpea mosaic virus (BlCMV). Biological and serological tests putatively identify the virus as a strain of BlCMV.

A831

A TOMATO SPOTTED WILT-LIKE VIRUS FROM VERBESINA ALTERNIFOLIA. I. Hayati, A. S. Kline, K. S. Kim and R. C. Gergerich. Dept. of Plant Pathology, Univ. of Arkansas, Fayetteville, AR 72701.

Inoculation of sap from yellow ironweed (Verbesina alternifolia) exhibiting mosaic symptoms resulted in necrotic local lesions in Chenopodium quinoa and Nicotiana rustica. Ultrastructural studies of these two hosts revealed large spherical particles (70-120 nm) similar to tomato spotted wilt virus

(TSWV). Unlike TSWV, the particles were individually enclosed in a second, external membrane which originated from either rough ER, the outer nuclear membrane or Golgi bodies. The particles were associated with two types of cytoplasmic inclusions distinct from those induced by known TSWV. ELISA tests indicated no serological relationship to common TSWV isolates or the impatiens isolate of TSWV described by Law and Moyer (Phytopathology 79:1157).

A832

TRANSLOCATION OF BARLEY YELLOW DWARF VIRUS-PAV-IL IN TOLERANT AND SUSCEPTIBLE SISTER OAT LINES. H. M. Fouly and C. J. D'Arcy, Department of Plant Pathology, University of Illinois, 1102 S. Goodwin Ave., Urbana, IL, 61801.

Translocation of barley yellow dwarf virus PAV-IL (BYDV-PAV-IL) in two pairs of sister oat lines tolerant and susceptible to the virus was measured by triple antibody sandwich enzyme-linked immunosorbent assay. Two-week-old oats, grown in aeroponic culture, were inoculated with viruliferous Rhopalosiphum padi L. Plants were collected at 2-day intervals and each dissected into four parts: the shoot and three root segments (basal, central and apical). Symptoms of BYDV-PAV-IL were observed only on susceptible oat lines 9 days after inoculation. Virus was detected in shoots and all root segments of each line from 2 to 15 days after inoculation. There were no differences in virus titers between either shoots or roots of tolerant and susceptible oat pairs. Virus titers were often higher in apical root segments than in those closer to the crown. For these oat lines, there is no evidence that differences in virus translocation are responsible for differences is susceptibility to BYDV-PAV-IL.

A833

ELIMINATION OF SWEET POTATO FEATHERY MOTTLE VIRUS FROM SWEET POTATO USING *IN VITRO* CULTURE OR PROPAGATION OF APICAL BUDS UNDER GREENHOUSE CONDITIONS. H.M. Griffiths & S.A. Slack, Dept. Plant Pathology, Cornell University, Ithaca, NY 14853.

Axillary buds (≈3.5 mm) from sweet potato plantlets cv. Georgia Red infected with sweet potato feathery mottle virus (SPFMV) were established *in vitro* on a medium supplemented with 20 mg/L ribavirin (MSR), heat treated under a 4-hr alternating 35°C light/31°C dark regime for 28 days, and tested for virus freedom using dot-blot ELISA. SPFMV freedom was confirmed after growth on ribavirin-free medium. Culturing buds from proximal regions on MSR resulted in the highest proportion (83%) of SPFMV-free plantlets. An alternative method for obtaining SPFMV-free plants was to excise apical buds (≈7.0 mm) from infected greenhouse plants, to induce rooting *in vitro*, and then to transfer plantlets to greenhouse conditions for continued growth. For cv. Georgia Red, 12% of plants established from apical buds were SPFMV-free and for cv. Jewel, 65%. These two studies show that SPFMV-free plants can be obtained either by *in vitro* culture procedures using ribavirin and heat or by excising apical buds from greenhouse plants which were rooted *in vitro* prior to transferring to the greenhouse.

A834

INCIDENCE OF BARLEY YELLOW DWARF VIRUSES (BYDV) IN WHEAT AND OTHER HOSTS IN ARKANSAS. T. Mahmood, R. C. Gergerich and C. J. D'Arcy. Depts. of Plant Pathology, Univ. of Arkansas, Fayetteville, AR 72701, Univ. of Illinois, Urbana, IL 61801.

Leaf samples of symptomatic wheat (Triticum aestivum L.) were collected from commercial plantings throughout Arkansas in the spring of 1989 for BYDV testing. Samples were assayed by indirect ELISA using polyclonal antisera for trapping and monoclonal antibodies for detection of the PAV, MAV, and RPV serotypes of BYDV. Of 588 wheat samples tested, 421 were positive for the PAV serotype, 13 for RPV, and one for MAV. Surveys of weed and crop plants during the summer of 1989 showed that fescue is a potential oversummering host for the PAV serotype of BYDV. Three-year old plots of 'Kentucky 31' fescue, which were free of the endophyte Acremonium coenophialum, had a significantly higher incidence of BYDV (86%) than endophyte-infected plots (42%).

A835

1989 SURVEY FOR THREE SEROTYPES OF BARLEY YELLOW DWARF VIRUSES IN OAT AND WHEAT FIELDS IN ILLINOIS. C. J. D'Arcy, Department of Plant Pathology, A. D. Hewings, USDA ARS, Crop Protection Research Unit, and C. E. Eastman, Natural History Survey, University of Illinois, 1102 S. Goodwin Ave., Urbana, IL, 61801.

Fifty leaf samples were collected in a random pattern from each of 11 oat and seven wheat fields in Illinois during May and June 1989. Samples were assayed for three serotypes of barley yellow dwarf viruses (BYDV) in two types of enzyme-linked immunosorbent assay (ELISA). Double antibody sandwich ELISA systems used polyclonal antibodies for virus detection; triple antibody sandwich ELISA systems used monoclonal antibodies for detection. Incidences of BYDV-PAV serotypes were 0-42% in oat fields and 0-2% in wheat fields; incidences of BYDV-RPV serotypes were 0-8% and 0-4% in oat and wheat fields, respectively. Only one BYDV-MAV serotype was detected. Results from the two BYDV-PAV ELISA systems were in agreement for over 98% of the 900 samples; however, there was less agreement between the two BYDV-RPV ELISA systems. Differences in disease incidence were noted among oat cultivars and across geographic regions of Illinois in 1989.

A836

EFFECT OF DPX-V9360 AND PRIMISULFURON IN MAIZE DWARF MOSAIC VIRUS A SEVERITY IN CORN. M. J. VanGessel[1], K. R. Zagula[2], S. A. Lommel[2], and H. D. Coble[1]. [1]Dept. of Crop Sci. [2]Dept. of Plant Pathology. North Carolina State University, Raleigh, NC 27695-7620

Greenhouse studies were conducted to evaluate the effect of the herbicides DPX-V9360 and primisulfuron on maize dwarf mosaic virus A (MDMV-A) infection in corn (*Zea mays* L. cv. Pioneer 3147). Both herbicides have been developed for post-emergence control of johnsongrass (*Sorghum halepense* L. (Pers)), an overwintering host for the virus. Either herbicide applied 24 hours after inoculation with MDMV-A suppressed visual symptoms when rated 17 days after treatment. Virus concentration, as determined by indirect ELISA, was significantly reduced when either herbicide was applied 24 hours before inoculation and tissue collected 17 days after treatment. Thus it appears either herbicide is reducing the severity of MDMV-A infection.

A837

SUGAR CONTENT, ANTHOCYANIN PRODUCTION AND REDDENING IN SUBTERRANEAN CLOVER RED LEAF (SOYBEAN DWARF) LUTEOVIRUS-INFECTED SUBTERRANEAN CLOVER LEAVES. A. E. Zipf and P. A. Hedin. USDA, ARS, Crop Science Research Laboratory, Mississippi State, MS 39762-5367.

Symptoms of subterranean clover red leaf (soybean dwarf) luteovirus infection of Trifolium subterraneum cvs. Mt. Barker and Geraldton include distinctive reddening of leaflets which develops from the margins inward. The bright reddening is restricted to the upper and lower epidermal cells. Increases in sucrose and fructose detected by gas chromatography coincided with increased reddening of infected Mt. Barker leaves. Feeding of detached Geraldton leaves with 5% solutions of fructose, sucrose, and glucose produced reddish-purplish discolorations within 5 days. There were no differences in RFs between anthocyanin pigments isolated from virus-infected leaves or sugar-fed leaves.

A838

DISEASES OF PEPEROMIA, IMPATIENS AND HIBBERTIA CAUSED BY CUCUMBER MOSAIC VIRUS. **Stanislaw Flasinski, Simon Scott, J. Q. Xia, Chao Sun, and O. W. Barnett, Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634-0377**

A disease of *Peperomia* characterized by large, black ringspots was caused by an isolate of cucumber mosaic virus (CMV-pep). Various *Peperomia* cultivars exhibited chlorotic sectoring, chlorotic ringspots, necrotic spots, and/or necrotic rings after inoculation with CMV-pep and most cultivars were stunted relative to uninfected plants. CMV-pep formed spurs in Ouchterlony serology tests with D and S serotypes. An S serotype isolate of CMV was found in *Impatiens hawkeri*-type with mosaic and strapleaf symptoms, the first of this serotype found in South Carolina. A mosaic disease of *Hibbertia scandens* in California also was due to an S serotype. Host reactions, agarose gel electrophoresis of virions and RNAs, and polyacrylamide gel electrophoresis of coat proteins and dsRNAs showed minor differences among the isolates.

A839

TOLERANCE OF Fusarium SPECIES TO HYGROMYCIN AND BENOMYL. L.R. Todd and T. Kommedahl, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

To study the feasibility of using the hygromycin and benomyl resistance genes as markers in Fusarium, 13 species (56 isolates) were tested for tolerance to hygromycin and 13 species (59 isolates) were tested for tolerance to benomyl. Growth was detected in 6 of the 13 species on a medium containing 100 µg/ml of hygromycin, while only 3 of these 6 grew on 150 µg/ml. Tests with a benomyl-amended medium revealed that all species grew at 1 µg/ml, 10 at 3 µg/ml and 4 at 5 µg/ml. All isolates grew more slowly on the selective medium than on unamended potato-dextrose agar and all 21 F. moniliforme isolates grew more slowly on

hygromycin-amended media compared with a transformed isolate containing the hygromycin resistance gene. The ability to grow on amended media appeared to be species-dependent. These results indicate that the genus Fusarium contains natural tolerance to both hygromycin and benomyl and that each isolate must be tested to determine if the use of these marker genes is suitable.

A840

A REVERSE GENETICS APPROACH TO CLONING A PHYTOALEXIN-DETOXIFICATION GENE FROM FUSARIUM SOLANI F.SP. PHASEOLI. D. Li, C. L. Schardl, and D. A. Smith. University of Kentucky, Lexington, KY 40546-0091.

Phaseolus vulgaris (French bean) produces three major phytoalexins, phaseollin, phaseollinisoflavan and kievitone, all of which are enzymically detoxified by the pathogen Fusarium solani f. sp. phaseoli (Fsp). The secreted glycoenzyme kievitone hydratase (KHase) has been purified and the N-terminal amino acid sequence determined. In order to identify clones of the khs gene, two genomic libraries were prepared. Fsp DNA was partially digested with Sau3A1 and fractionated by rate zonal centrifugation. Fragments of 8-23 kb were ligated into λFIXII phage vector to produce a library of 5 x 10^5 primary plaques. Fragments of 30-42 kb were ligated into cosmid pKBY2, an E. coli/A. nidulans shuttle vector, giving rise to 5 X 10^5 colonies. Oligonucleotide mixtures, based on the KHase N-terminus, are being used as probes. Putative positives will be analyzed by Southern blot and by heterologous expression in KHase-deficient fungi.

A842

LIGHT REGULATED GENE EXPRESSION IN CERCOSPORA KIKUCHII. M. Ehrenshaft, J.A. Rollins, D.C. Walker, R.G. Upchurch. North Carolina State University, Raleigh, NC 27695-7616.

Production of the phytotoxic polyketide, cercosporin, is light induced in the fungal soybean pathogen Cercospora kikuchii. In order to identify genes and gene products involved in toxin synthesis, in vitro translation products of poly A⁺ RNA extracted from light and dark grown cultures and from light-grown toxin-minus mutants were analyzed by two dimensional gel electrophoresis. Several light enhanced polypeptides were identified from the wild type strain that were not detected from light-grown, toxin-minus mutants. Genes whose expression is light enhanced were also identified from a cDNA library. The characterization of these clones is underway.

A843

COMPLETE NUCLEOTIDE SEQUENCE OF SEVERAL DOUBLE-STRANDED RNA SPECIES ASSOCIATED WITH HYPOVIRULENCE OF THE CHESTNUT BLIGHT FUNGUS. R. Shapira, G.H. Choi, B.I. Hillman and D.L. Nuss, Dept. of Mol. Oncology and Virology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

The complete nucleotide sequence of several double-stranded RNA species associated with the hypovirulent strain EP713 of the chestnut blight fungus, *Cryphonectria parasitica*, was determined by analysis of a series of overlapping and full-length cDNA clones. The deduced RNA sequence of the major large double-stranded was 12,716 bp in length excluding the terminal polyA:polyU homopolymer domain. Four putative large open reading frames were found within the polyA-containing strand. The predicted locations of initiation and termination codons of each ORF were confirmed by *in vitro* expression studies. In addition, sequence analysis of cDNA clones of several small (~700 bp) dsRNA species revealed them to be derived from the largest dsRNA by internal deletion events. To our knowledge, this represents the first report of the complete sequence of a double-stranded RNA genetic element associated with altered virulence of a plant pathogenic fungus.

A844

IDENTIFICATION AND CHARACTERIZATION OF GENOMIC CLONES ENCODING ENDOTHIAPEPSIN OF *Cryphonectria parasitica*. G.H. Choi, R. Shapira, B.P. Rae and D.L. Nuss, Dept. of Mol. Oncology and Virology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

Endothiapepsin is a secreted aspartic proteinase produced by the chestnut blight fungus *Cryphonectria parasitica*. The gene encoding endothiapepsin was identified by screening a *C. parasitica* genomic library with a probe generated from *C. parasitica* DNA by the polymerase chain reaction (PCR) employing two synthetic oligonucleotides which corresponded to portions of the known endothiapepsin amino acid sequence. Nucleotide sequence analysis revealed perfect agreement with the experimentally derived 330 amino acid sequence of mature endothiapepsin, three introns, a putative 88 amino acid preproenzyme sequence and characteristic upstream CAAT and TATA motifs. Codon usage for endothiapepsin was similar to that of rhizopuspepsin and human pepsinogen. The availability of the genomic clone of this well characterized proteinase provides opportunities for investigating structure-function relationships of aspartic proteinases and regulation of gene expression in *C. parasitica*.

A845

CHARACTERIZATION OF EXTRACELLULAR ENZYMES OF COLLETOTRICHUM SPECIES. R. S. Redman, A. Schlemmer, and R. Rodriguez, Department of Plant Pathology, University of California, Riverside, CA 92521

Extracellular protease and endoglucanase from *C. lindemuthianum, C. coccodes, C. acutatum, C. frigariae, C. gloeosporoides*, C. musae, and *C. graminicola* were characterized. A high degree of variation was observed in the biochemical regulation of these enzymes among different isolates of each species. Four active forms of the endoglucanase were separated by SDS-PAGE. One of the active forms of endoglucanase (EG-1) was purified to homogeneity and found to be an O-linked glycoprotein. An oligonucleotide probe was constructed based on a partial protein sequence from EG-1; genomic clones were isolated by hybridization to the oligonucleotide probe. Characterization of genomic clones for EG-1 and regulation of the enzyme will be discussed.

A846

A RECIPROCAL TRANSLOCATION ASSOCIATED WITH HOST-SPECIFIC VIRULENCE IN *COCHLIOBOLUS HETEROSTROPHUS*. H. Chang and C. R. Bronson. Department of Plant Pathology, Iowa State University, Ames, IA, 50011.

The host-specific virulence of race T of *Cochliobolus heterostrophus* to T-cytoplasm maize is controlled by the locus *Tox1*. We have tested the hypothesis that race T (tox+) and race O (tox-) isolates of *C. heterostrophus* differ by a chromosome rearrangement with its breakpoint at or near *Tox1*. The chromosomes of 16 near-isogenic laboratory strains (8 tox+ and 8 tox-) and 16 unrelated field isolates (8 tox+ and 8 tox-) were separated by pulsed field gel electrophoresis and hybridized with probes known to map around *Tox1*. The hybridizations demonstrated that the two chromosomes associated with *Tox1* in the tox+ isolates are reciprocally translocated with respect to their homologous chromosomes in the tox- isolates. In addition, the tox+ field isolates had fewer chromosome size polymorphisms than the tox- field isolates. These results suggest that race T may have evolved from race O by a reciprocal translocation event.

A847

ANALYSIS OF THE MOLECULAR KARYOTYPE OF THE WHEAT BUNT PATHOGENS *TILLETIA CARIES* AND *T. CONTROVERSA*. B. W. Russell*, and D. Mills*+. Dept of Botany and Plant Pathology+, Genetics Program*. Oregon State University, Corvallis, OR. 97331-2902.

Tilletia caries and *T. controversa* are the causal agents of common and dwarf bunt of wheat, respectively. We are studying strains from single sporidia of the fungi (presumed to be haploid), and of F1 hybrid strains resulting from a cross of *T. caries* X *T. controversa*. Genetic relatedness of these strains is being determined by molecular karyotypic analysis using CHEF gel electrophoresis. A method to obtain intact chromosomes has been developed that eliminates the need for protoplast formation. An identical karyotype was observed for two strains of *T. caries*. Each strain had 15 chromosome-sized bands which ranged in size from approximately 880 Kilobases(Kb) to 4,000 Kb. Ten to 12 bands were observed in 4 strains of *T. controversa* and their sizes ranged from ca. 990 Kb to 3,900 Kb. The chromosome-sized DNA bands of 3 F1 hybrid strains numbered 10 to 12 and were from ca. 770 Kb to 3,500 Kb in size. Some variability in band number may be due to unresolved doublets and chromosome length polymorphisms. Similar sized DNA bands are present in strains of both fungi and in the F1 hybrid strains.

A848

IDENTIFICATION OF A GENE ENCODING PISATIN DEMETHYLATING ABILITY FROM *FUSARIUM OXYSPORUM* F. SP. *PISI*. L. M. Delserone and H. D. VanEtten, Departments of Plant Pathology, Cornell University, Ithaca, NY 14853 and University of Arizona, Tucson, AZ 85721, respectively.

Previous studies have established that the ability to rapidly demethylate, and thereby detoxify, the phytoalexin pisatin is required by *Nectria haematococca* for high virulence on garden pea (*Pisum sativum*). Preliminary studies suggest that this also is true for *Fusarium oxysporum* f. sp. *pisi*. To determine whether there is a relationship between genes encoding pisatin demethylating ability (pda) in both fungi, genomic DNA of *F. oxysporum* was probed with a cloned PDA gene from *N. haematococca*. A gene with similarity to that from *N. haematococca* was identified and cloned. Sequence analysis of the gene from *F. oxysporum* will facilitate its further study by expression in a heterologous system and by gene-disruption, experiments which can determine the role of the gene in the pathogenicity of *F. oxysporum*.

A849

SELECTION OF PROBES SPECIFIC FOR SPECIES OR ISOLATES OF THE GENUS Pythium. Frank N. Martin, Plant Pathology Department, University of Florida, Gainesville, Fla. 32611

Selection of probes specific for species or isolates of the genus Pythium may be aided by identification of unique DNA sequences in the mitochondrial DNA (mtDNA). The circular mitochondrial genome ranges in size from 59 to 73 kb and is arranged as a large inverted repeat representing 71 to 83% of the genome, with the repeats separated by a small and a large unique region. Comparison of mtDNA restriction maps of different isolates of P. oligandrum indicate that the small unique region is the most variable portion of the genome, with insertions/deletions accounting for a size variation of 0.92 to 3.92 kb. Construction of a detailed restriction map of this region and selection of the appropriate fragments provided probes that were specific for isolates sharing the same restriction map. These fragments also are useful as species-specific probes as indicated by hybridization studies with 25 other Pythium spp.

A850

DEVELOPMENT OF STEWART'S WILT ON SEQUENTIAL PLANTINGS OF SWEET CORN. P. Fallah Moghaddam, J. A. Hawk, Plant Science, Univ. of Delaware, Newark, and J. K. Pataky, Plant Pathology, Univ. of Illinois, Urbana.

Six sweet corn hybrids partially resistant, intermediate, or susceptible to Erwinia stewartii were planted on four dates in Newark, DE; St. Louis, MO; and Urbana and Rochelle, IL. Natural occurrence of Stewart's wilt was assessed. Incidence (%) was measured throughout the season. Severity was rated from 1 to 9 about 2 wk before harvest. Stewart's wilt did not occur at Rochelle. At the other locations, reactions of hybrids could be determined from incidence or severity. Severity was similar among locations, ranging from 5 to 7.5, 3 to 5 and 1 to 3.8 for susceptible, intermediate, and resistant hybrids, respectively. Incidence varied among locations. At Urbana, final incidence was similar among plantings, ranging from 98-100%, 56-100%, and 22-51% for susceptible, intermediate, and resistant hybrids, respectively. Final incidence was lower at Newark and St. Louis than Urbana. Incidence decreased with each planting at Newark. Incidence was similar among plantings at St. Louis.

A851

NEW GEMINIVIRUS EPIDEMIC IN FLORIDA TOMATOES AND PEPPERS. G.W. Simone*, J.K. Brown**, E. Hiebert*, and R.E. Cullen*. Plant Pathology Dept.*, Univ. of Florida, Gainesville, FL 32611 and the Dept. of Plant Pathology**, Univ. of Arizona, Tucson, AZ 85721.

Tomato plant samples, received in the Florida Extension Plant Disease Clinic in September 1989, exhibited leaflet curl and distortion and either a bright yellow mosaic or a mottled, interveinal chlorosis. Light microscopy of various tissues (azure A–stained) revealed nuclear inclusions in phloem cells of leaflets and petioles characteristic of geminiviruses. Infected tissues tested positive in moderately stringent hybridization assays with DNA probes for the A components of bean golden mosaic, chino del tomate and tomato golden mosaic viruses. The viral disease was widespread throughout the west–central and southwest tomato production areas, where incidence ranged from 5–>95%, with highest prevalence occurring in early August plantings. Although affected peppers (found in one location) exhibited no foliar symptoms, fruit were misshapen and exhibited longitudinal color breaking and pod necrosis. These pepper samples tested positive for a geminivirus in cytological studies and in hybridization studies using BGMV and CdTV probes.

A852

EARLY POWDERY MILDEW OF GREENHOUSE-GROWN TOMATOES IN FRANCE. Burgerjon, A., Nicot, P.C., Bertrand, F. and Blancard, D. Station de Pathologie Végétale, INRA, Domaine Saint Maurice, 84140 Montfavet, FRANCE.

A powdery mildew (tentatively *Erysiphe* sp.) was observed on tomatoes in France in 1988. It is now found in greenhouses in most of the tomato-growing areas of the country, where it can affect plants early in the season. In host range studies, the fungus infected and sporulated on the foliage of *Lycopersicon esculentum* (all varieties tested), *Solanum melongena*, *S. tuberosum* and *Nicotiana tabacum* "Xanthi", and on cotyledons of *Cucumis sativus*, *Lagenaria leucantha* and *Helianthus annuus*. Single-spore clones of the pathogen are conveniently maintained in axenic culture on detached tobacco leaves or cucumber cotyledons. Isolates of the tomato powdery mildew were distinguished from cucurbit isolates of *Erysiphe cichoracearum* by the shape of conidia and the kinetics and abundance of sporulation. Single-spore isolates of tomato powdery mildew were unable to form perithecia when paired with complementary testers of *E. cichoracearum* isolated from cucurbits.

A853

EFFECT OF PRE- AND POST-PLANTING ENVIRONMENT ON POTATO SEEDPIECE DECAY. R. V. James and W. R. Stevenson, Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706.

Seedpiece decay, decrease in plant stand, and loss of plant vigor have been persistent, serious problems affecting Wisconsin's potato crop. These problems have often been associated with bacterial soft rot of the planted seedpiece caused by *Erwinia carotovora* subsp. *atroseptica* (*Eca*). Procedures that enhance the natural ability of potato tubers to heal, decrease the potential for bacterial seedpiece decay and the need for chemical treatments. The effects of pre-plant treatments on seedpiece decay were evaluated under growth chamber conditions using three soil temperatures and four soil moistures. Pre-plant treatments included combinations of pre-cutting storage temperature (4-18° C) and temperature and duration for wound healing between cutting and planting (0-5 days at 13 or 18° C). Experiments were conducted with inoculated (10^7 CFU/ml *Eca*) and uninoculated Atlantic and Russet Burbank seedpieces planted in silt loam or sandy loam soils. Emergence (rate and percent), severity of seedpiece decay, and mean fresh weight per shoot were compared. In general, decay was least with moderate soil moisture and with precut, wound-healed seedpieces and was greatest when soil was saturated after planting, regardless of soil type, cultivar, pre-plant treatment, or growth temperature. There was no consistent effect of pre-cutting temperature on seedpiece decay. Additional treatment combinations are being tested in growth chamber and field experiments.

A855

IDENTIFICATION OF VIRUS DISEASES OF CUCURBITS ON GUAM. L.S.Yudin, G.C.Wall, R.J.Quitugua, M.W.Johnson & J.Cho. College of Agriculture & Life Sciences, University of Guam, Mangilao, GU, 96923, and College of Tropical Agriculture & Human Resources, University of Hawaii, Honolulu, HI, 96822.

Viral diseases are a common problem on cucurbits, the most important cash crops on Guam. In order to control these, it was necessary to identify them. Antisera for WMV1 (syn. PRSV-W), WMV2, CMV, and ZYMV were obtained from D. Gonsalves (Cornell University). Positive IDs were made via ELISA for WMV1, CMV, and ZYMV. The latter predominated in watermelon samples from the northern sector of the island, while WMV1 predominated in southern samples. WMV1 was also found on zucchini squash, cantaloupe, and in the common weeds Luffa acutangula, Momordica charantia, and Carica papaya. CMV was found on a native weed, Achyranthes cansescens (Amaranthaceae). Aphid vectors found in watermelon fields were Aphis gossypii and A. craccivora.

A856

DECLINE OF SIBERIAN ELM ON THE GREAT PLAINS.
R. W. Stack[1], J. M. Krupinsky[2] and J. A. Walla[1]. 1) North Dakota State Univ., Fargo, ND 58105 and 2) USDA-ARS Northern Great Plains Res. Sta., Mandan, ND 58554.

Since the dust bowl days of the 1930's, Siberian elm (*Ulmus pumila*) has been widely planted on the Great Plains, both in single- and multi-row field windbreaks to control soil erosion and in farmstead shelterbelts. Many plantings are now in decline. Predisposing factors include drought, insect defoliation, herbicide injury and winter damage. Stem cankers caused by *Tubercularia ulmea* and *Botryodiplodia hypodermia* are often the proximate cause of dieback and death. Trees in single-row field windbreaks are affected at a younger age and show more severe damage than those in larger plantings, possibly because these are more exposed to the predisposing agents.

A857

MOVEMENT OF A CYTOPLASMIC HYPOVIRULENCE AGENT IN CHESTNUT BLIGHT CANKERS. L. Shain and J. B. Miller. Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Chestnut blight cankers initiated in the spring with a virulent (V) methionine auxotrophic (met^-) strain of Cryphonectria parasitica were converted to hypovirulence by placing one or two discs of agar and mycelium of a cytoplasmic hypovirulent (CH) non-auxotrophic strain in bark wounds at the base of cankers ca. 8 wk. later. Cankers collected with increasing time after introduction of CH inoculum were monitored for movement of the CH agent by culturing cirrhi and bark discs on met^+ or met^- nutrient media. Movement was confirmed by an isolate exhibiting the typical morphology of the introduced CH strain on met^+ but little or no growth on the met^- media. Cultures from bark showed that the CH agent moved through mycelium around the canker periphery within 3-6 wk. after its introduction at the canker margin. Conversion of the canker interior proceeded more slowly. Cirrhi, however, continued to yield V, met^- cultures up to 16 months later, even though underlying bark yielded CH, met^- cultures. A reluctance of CH agents to enter the asexual sporogenic apparatus in cankers may contribute substantially to ineffective CH dissemination.

A858

CHARACTERISTICS OF OAK DECLINE SITES WITHIN THE MISSISSIPPI RIVER DRAINAGE SYSTEM. **F. I. McCracken[1], V. D. Ammon[2], T. H. Filer[1], T. E. Nebeker[2], R. Wolf[3], H. E. Kennedy[1] and J. D. Solomon[1]. [1]USDA For. Serv., Stoneville, MS 38776, [2]Miss. State Univ., Miss. State, MS 39762, and [3]NOAH/Natl. Weather Serv., Stoneville, MS 38776.**

Tree, site, and soil measurements taken from oak decline plots in Missouri, Kentucky, Arkansas, and Mississippi indicate that decline sites have lower site indices; greater basal area; more red oaks; slower growing, smaller trees; greater mortality; and higher incidences of insects and disease. Growth ring analysis showed the effects of previously recorded droughts, floods, and winter storms. Mean basal area increment (BAI) was also used to differentiate healthy from decline plots. Decreases in BAI in decline plots appear to be correlated with lower summer rainfall and lower mean winter temperatures. These data suggest that long term climatic trends accompanied by shorter term environmental and biological stress factors are involved in hardwood decline and mortality.

A860

INTERACTION OF ASPEN AND GYPSY MOTH. John H. Hart, Depts., Botany & Plant Pathology and Forestry, Michigan State University, East Lansing, 48824.

In 1986 studies were initiated to determine the effects of gypsy moth (Lymantria dispar) defoliation on bigtooth (Populus grandidentata) and quaking aspen (P. tremuloides) in Michigan. Six areas of mixed northern hardwoods of approximately 20 acres each were selected in Midland County, an area of expected high defoliation. In late June, species, DBH, % defoliation and condition were recorded for each tree (75 bigtooth and 113 quaking aspen). Average 4-year defoliation of quaking and bigtooth aspen was 37% and 18%, respectively. Mortality (1987-1988) of quaking aspen (8%/yr) was 4 times higher than that of bigtooth aspen (2%/yr). Armillaria sp. occurred on 91% of the dead trees. Only trees with 80% or more defoliation in 1986 died in 1987 or 1988. In 1989 tree mortality was not correlated with 1986 defoliation but with at least one year of defoliation greater than 70% during the preceding 4 years. Stems coded sub-dominant in 1986 died at 2 to 3 times the rate of stems coded co-dominant or dominant, although 1986 defoliation rates were similar.

A863

SUGAR MAPLE HABITAT CLASSIFICATION AS PART OF A HARDWOOD DECLINE SURVEY IN WISCONSIN. M.E. Mielke[1], C.L. Rezabek[2], J. Cummings Carlson[2], and A.J.R. Gillespie[3]. [1]USDA Forest Service, NAFPM, St. Paul, MN 55108 and [2]Wisconsin Department of Natural Resources, Madison, WI 53707, and [3]USDA Forest Service, Methods Application Group, Ft. Collins, CO 80524.

The health of sugar maple and associated northern hardwoods was surveyed across an air quality gradient (rainfall, pH, nitrate and sulfate) in northern Wisconsin. A habitat type classification system (Kotar, et al., 1988) was used to evaluate site quality and its possible effect on the health of sugar maple. Of the 96 ground plots evaluated, 40% were mesic forests with very rich soil nutrients, 43% mesic forests with rich soil nutrients, and 4% were dry mesic forests with medium soil nutrients. Approximately 105 sugar maple trees per acre occurred on mesic forest sites with very rich soil nutrients. Of these trees, 98% were healthy and 2% were dead or declining. The sites considered optimal habitat for sugar maple had the largest number of healthy trees and the suboptimal sites had the fewest number of sugar maple present.

A864

CHARACTERIZATION OF OAK DECLINE IN THE TENNESSEE-TOMBIGBEE RIVER BASIN. [1]Vernon D. Ammon, [2]Francis I. McCracken, [1]T. Evan Nebeker, [2]Ted H. Filer, and [2]Jim D. Solomon. [1]Department of Plant Pathology and Weed Science, Mississippi State, MS and [2]USDA-USFS Southern Hardwood Laboratory, Stoneville, MS.

Biological, environmental, and edaphic data collected from sites with declining oaks in the Tennessee-Tombigbee River basin were compared to data collected from an equal number of sites where oak decline was lacking. Decline sites contained primarily dominant and co-dominant trees whose growth had slowed, which had poorer form, were lower in grade, smaller in diameter, and which were taller and older than trees on control plots. Declining trees were evenly distributed among the eight topographic positions evaluated. Discriminant analysis procedures are being used to develop a hazard rating system for oak decline in the South.

A865

A SYNTHESIS FROM STUDIES ON DECLINE AND MORTALITY OF CHAMAECYPARIS NOOTKATENSIS IN SOUTHEAST ALASKA. P.E. Hennon, USDA Forest Service, Juneau, AK 99802, C.G. Shaw III, USDA Forest Service, Fort Collins, CO 80526, and E.M. Hansen, Oregon State University, Corvallis, OR 97331.

Decline is extensively distributed on 200,000 ha of undisturbed forest throughout SE Alaska. Dating mortality, historical records, and early aerial photographs suggest that decline began about 1880. Decline is associated with poorly drained soils where mortality has continued since onset and regeneration is sparse or absent. Spread of decline to new sites is not apparent, but local encroachment (<1m/yr) affects adjacent forest along a gradient from bog to better drainage. Symptoms on Chamaecyparis nootkatensis, the principle victim of decline, include dead fine roots, necrotic lesions on coarse roots and boles, slowed radial growth, and thinning or yellowing of crowns. Over 50 taxa of fungi, nematodes, and bark beetles are associated with dying cedars, but none can kill healthy trees. Natural, abiotic factors are likely the primary cause of this extensive forest decline.

A866

Transmission of *Leptographium procerum* to eastern white pine, *Pinus strobus*, seedlings by the pales weevil, *Hylobius pales*. R. J. Nevill and S. A. Alexander, Department of Plant Pathology, Physiology & Weed Science, VPI&SU, Blacksburg, VA 24061-0330.

Field collected pales weevils, *Hylobius pales*, artificially infested with spores of *Leptographium procerum* were individually caged on 20 eastern white pine, *Pinus strobus*, seedlings and allowed to feed for 24 h. As a control, another 20 field collected weevils not artificially infested with *L. procerum* were individually caged and allowed to feed for 24 h on eastern white pine seedlings. To compare transmission rates 20 eastern white pine seedlings were inoculated with 5 X 10 mm agar plugs containing *L. procerum*. Another 20 seedlings were mock inoculated with sterile agar as controls. After six months, *L. procerum* could be recovered from all of the seedlings fed on by weevils artificially inoculated with the fungus. *L. procerum* was recovered from 11 of the 20 seedlings fed on by weevils not artificially infested with the fungus. The fungus was recovered from all of the seedlings inoculated with agar containing *L. procerum*. These results illustrate that *L. procerum* can be transmitted by the pales weevil and supports observations that this insect is a vector of the pathogen.

A867

SCREENING LARCH IN VITRO FOR RESISTANCE TO MYCOSPHAERELLA LARICINA. M. E. Ostry, P. M. Pijut, and D. D. Skilling, USDA Forest Service, North Central Forest Experiment Station, 1992 Folwell Avenue, St. Paul, MN 55108.

Mycosphaerella laricina causes a serious needlecast disease of European larch (Larix decidua) in the north-central and northeastern United States. Resistance among seed sources varies; some trees are so susceptible that they die after repeated defoliations. A system using tissue culture was developed to rapidly screen larch for resistance to infection by M. laricina. Adventitious shoots produced from six larch seed sources were inoculated with mycelium of M. laricina. Disease severity varied among seed sources, and rankings after 6 weeks correlated with results from previous field screening trials. Conidiomata with conidia of M. laricina developed on the most susceptible larch. Tissue culture and in vitro screening offer the possibility of determining relative resistance of larch selections so that resistant larch can be recommended for planting.

A868

INCIDENCE AND PATHOGENICITY OF SEEDBORNE FUSARIUM ON DOUGLAS-FIR (PSEUDOTSUGA MENZIESII (MIRB.) FRANCO). P.E. Axelrood, D. Fong, and R. Radley. B.C. Research Corporation, Forest Biotechnology Centre, Vancouver, B.C., V6S 2L2, Canada.

Fusarium has been associated with an increased incidence of disease in B.C. conifer nurseries. To determine if Fusarium inoculum is introduced on conifer seed, 10 B.C. Douglas-fir seedlots were assessed. Fusarium was found on 0.25% to 10.75% of the seeds in half of the seedlots. Incidence of Fusarium was not correlated with seedlot age or percent germination. Species isolated were F. acuminatum, avenaceum, lateritium, moniliforme, oxysporum, poae and sambucinum. Twelve isolates (6 species) were assessed for pathogenicity. F. oxysporum isolates were the most pathogenic followed by F. monilforme. F. lateritium caused a low incidence of disease but was ineffective when tested for biological control of F. oxysporum.

A869

FUNGI ASSOCIATED WITH NURSERY TREE SEEDLINGS IN HAITI. G. B. Runion, W. D. Kelley, and R. K. Reid. School of Forestry, Auburn University, AL 36849 and SECID/Auburn University Haiti Agroforestry Research Project, Petion-Ville, Haiti.

Twenty-four nurseries throughout Haiti were visited and disease symptoms were observed on seedlings from 18 genera of trees. Diseased tissues were collected, cultured in moist incubation chambers and/or on agar media and identified to genus. Fusarium spp. and Rhizoctonia spp. were the most prevalent fungi associated with seedlings exhibiting symptoms of damping-off. Leaf spots were common on many genera of trees and were most often associated with Cercospora spp., Alternaria spp. and Pestalotia spp. Anthracnose symptoms were consistently associated with Colletotrichum spp. Overall, disease levels in Haitian nurseries were low, although diseased seedlings generally exhibited symptoms of several types of disease and were associated with a large number of fungi. Future efforts on seedling diseases in Haitian nurseries will center on disease etiology.

A870

FAMILY PERFORMANCE OF WESTERN WHITE PINE IN FIELD AND BLISTER RUST INOCULATION TESTS. R.S. Hunt and M.D. Meagher, Forestry Canada, Pacific Forestry Centre, 506 West Burnside Rd., Victoria, B.C. V8Z 1M5.

Eighteen open pollinated families of western white pine (Pinus monticola D. Don) were established in each of two plantations. Tallies of white pine blister rust (Cronartium ribicola Fisch.) cankers at 12 years revealed that three of the four least-cankered families came from canker-free parent trees. Seedlings of six families from canker-free parents were inoculated. Needle spotting incidence was not correlated with plantation cankering (Spearman's rank value = 0.08), but second year cankering was (Spearman's rank value = 0.75). Although the canker incidence from inoculation of the best two field performing families was lowest (84 and 87%), the comparable incidence on the worst field performing family was not greatly different (89%). It was difficult to pick the best families from seedling inoculation data, but such data could be used to cull some poor families.

A871

HISTOPATHOLOGY OF YEAR-OLD CORNUS FLORIDA L. INOCULATED WITH DISCULA SP. C. H. Walkinshaw, and R. L. Anderson. USDA Forest Service, Resistance Screening Center, Rt. 3 Box 1249-A, Asheville, NC 28806.

Dogwood anthracnose has increased dramatically in the eastern United States within the last decade. Diagnostic symptoms include small leaf spots that enlarge to form blotches of necrotic tissues. Our study reproduced these foliar symptoms in the greenhouse using techniques previously published from this laboratory. In this study leaves were inoculated with 2000 spores per milliliter of Discula sp., fixed, embedded and stained for microscopy. Intracellular and intercellular hyphae were abundant in the epidermis, mesophyll and leaf vascular tissues. Extensive necrosis of palisade and spongy parenchyma cells seemed to precede fungus proliferation. Fruiting bodies of the pathogen were evident. These results confirm macroscopic observations that Discula sp. is highly invasive to dogwood.

A872

USE OF A LEAF DISK METHOD TO DETERMINE AGGRESSIVENESS OF *SEPTORIA MUSIVA*. J. M. Krupinsky. USDA, Agriculture Research Service, Northern Great Plains Research Laboratory, P.O. Box 459, Mandan, ND 58554

High and low aggressive isolates of *Septoria musiva* obtained from *Populus* leaves and cankers (Phytopathology 79:413-416) were compared on leaf disks obtained from four field grown *Populus* clones. One high and one low aggressive isolate were compared in seven studies. Nine leaf disks (three inoculated with a high aggressive isolate, three with a low aggressive isolate, and three with distilled water) were placed in wells in water agar in each petri dish. Clones reacted (percentage necrosis) significantly different from one another in all studies. Northwest was the most susceptible clone. Based on percentage necrosis of the leaf disks there were significant differences among isolates in all studies. The high aggressive isolates caused significantly more symptoms in 5 out of 7 comparisons. In general, high aggressive isolates can be separated from low aggressive isolates in leaf disk inoculations of field-grown leaves.

A876

PATHOGENICITY OF XYLELLA FASTIDIOSA TO AMERICAN ELM. JAMES L. SHERALD, Center for Urban Ecology, National Park Service, 1100 Ohio Dr. S.W., Washington, D.C. 20242.

American elm seedlings (4-mo-old, 20 cm ht) were inoculated in August 1988 with a strain of X. fastidiosa isolated from a naturally infected elm. Ten seedlings were inoculated with 0.025 ml of a bacterial suspension ($7x10^{7}$ cells/ml) in 3 scalpel wounds in the stem. Control seedlings were treated with buffer. By June 1989 all inoculated seedlings had developed leaf scorch symptoms which progressed from older to younger leaves and exhibited the undulating marginal necrosis typical of naturally infected trees. Controls remained symptomless. Terminal elongation was reduced by 32% and the stem caliper of treated and control seedlings was 0.48 cm and 0.70 cm respectively 14 months after inoculation. Bacteria characteristic of X. fastidiosa were isolated from 6 of the 10 inoculated seedlings but not from controls. Isolated strains gave a positive ELISA reaction for X. fastidiosa.

A877

SEM STUDIES OF THE DOGWOOD ANTHRACNOSE FUNGUS. S.C. Redlin, USDA-ARS, SBML, Beltsville, MD 20705-2350.

The dogwood anthracnose fungus (Discula sp.) causes lethal cankers on flowering dogwood, Cornus florida L., in eastern North America. A technique of removing leaf discs (17 mm dia.) from healthy dogwoods in the field, placing the discs in wells made in water agar media, wounding them with a heated metal rod, and inoculating them at the burned spot was useful for studying conidiomata. Conidiomata developed as subepidermal swellings below the two-armed trichomes. Mature conidiomata containing phialidic conidiogenous cells and conidia were observed six days after inoculation. Ostioles did not develop. Ellipsoid conidia were released through an irregular rupture of the conidiomatal wall. Ruptures sometimes occurred as a result of secession of the associated trichome.

A879

TEMPERATURE SENSITIVE NODULATION OF THE PRIMITIVE PEA CULTIVAR *IRAN* BY *RHIZOBIUM LEGUMINOSARUM* BV. *VICIAE* STRAIN PF2. W. Derrick[1], D. Kluepfel[1], and T.-A. Lie[2], Clemson University, SC[1] and Agricultural University, Wageningen, The Netherlands[2].

Nodulation of *Iran* by *R. leguminosarum* strain PF_2 is temperature mediated. The influence of temperature is strain specific and inherited in pea as a single dominant gene. Time after inoculation and region of root tissue where sensitivity to temperature change is greatest were determined. Temperature-switching in increments of 24 hours showed that inhibitory effects of non-permissive (20 C) and stimulatory effects of permissive (26 C) temperatures are greatest 3-4 days after inoculation. Root tip marking showed that nodules formed in the same relative position regardless of temperature treatment, indicating that the reduction in nodule formation is not due to delay in onset of infection. Roots grown and inoculated at 20 C show longitudinal splitting and peeling of epidermal cell layers similar to HR in aerial portions of incompatibly infected tissue. This response is absent in uninfected roots at either temperature or in infected roots at 26 C.

A880

Charles P. Woloshuk and Theo C. Verwoerd. Antifungal activity of chitinases expressed in transgenic tobacco. MOGEN International N. V. Einsteinweg 97, 2333 CB Leiden, The Netherlands

Nicotiana tabacum SR1 was transformed with a vector containing both an acidic chitinase gene from petunia and a basic chitinase gene from tobacco. The genes were expressed constitutively using the 35S promoter of Cauliflower Mosaic Virus. Two of the resulting transgenic plants showed high levels of expression of both genes in Northern analysis. This elevated expression was also observed at the protein level by Western analysis. Chitinase activity in these plants was 4 and 7 times that of the nontransformed tobacco, respectively. Total protein extracts were used to measure in vitro antifungal activity against Trichoderma viride and Fusarium solani. Extracts caused lysis of hyphal tips and inhibition of growth. In these assays the transgenic plants were 5 and 10 times, respectively, more active against these fungi than the controls. These data demonstrate the expression of active chitinases in transgenic tobacco and indicate that antifungal activity correlates with the level of enzyme activity.

A881

MONENSIN INHIBITION OF HAUSTORIAL FORMATION IN POWDERY MILDEWED BARLEY IS OVERCOME BY CALCIUM. H. H. Edwards Dept of Bio Sci, West. Illinois U., Macomb, Il 61455

Monensin will completely inhibit primary haustorial formation in primary barley leaf segments floating in 30 mM solution for 24 hr after inoculation. Leaf segments in water have appressoria 30% efficient at forming haustoria and segments in 10mM calcium are 50% efficient. The optimum application for both monensin and calcium is 8-12 hr after inoculation. It is during this time period that appressoria form and attach to the host epidermal wall but prior to infection peg development. Leaf segments floating in both monensin and calcium are 20% efficient. It is suggested that high host cytoplasmic calcium prior to infection peg development favors haustorial formation. Monensin, which disrupts N_a/H gradients, may inhibit release of stored calcium which is partially overcome by high external calcium.

A882

GERMINATION AND INFECTIVITY OF PHYTOPHTHORA INFESTANS IN THE PRESENCE OF FATTY ACIDS. Y. Cohen, Bar-Ilan University, Israel, U. Gisi, and E. Mösinger, Sandoz Agro Research, Switzerland.

Stable, water-sonicates of five unsaturated fatty acids (Sigma) were tested for their effects on both germination of Phytophthora infestans (isolate S49) in vitro and infection of potato (cv. Bintje) leaf discs at 15 C. Oleic, linoleic, arachidonic and eicosapentaenoic (EP) acid did not affect zoospore discharge at concentrations of up to 16.5 mM. Zoospore germination, however, was inhibited by linolenic, EP, arachidonic and linoleic acid, with ED_{50} values of about 1.65, 16.5, 16.5 and 33 µM, respectively. Oleic acid stimulated zoospore germination at 16.5 mM. Arachidonic and EP acid induced a strong necrosis in potato leaf discs at $\geqslant$ 1.65 mM, linoleic and linolenic acids did so at $\geqslant$ 16.5 mM. Oleic acid did not induce necrosis at 16.5 mM or below. Disease development was completely inhibited by linolenic, arachidonic and EP acid at 16.5, 165 and 165 µM, respectively, and partially by linoleic acid at 1.65 mM. Oleic acid stimulated lesion development and sporulation at 16.5 mM.

A883

ISOLATION AND STRUCTURES OF ACT-TOXIN I AND II, HOST-SPECIFIC TOXINS FROM THE TANGERINE PATHOTYPE OF ALTERNARIA ALTERNATA. K. Kohmoto, Y. Itoh, M. Kodama, H. Otani, and S. Nakatsuka*, Plant Pathology Lab., Fac. of Agric., Tottori University, Tottori 680, Japan, and *Organic Chemistry Lab., Fac. of Agric., Nagoya University, Nagoya 464, Japan.

ACT-toxin I and II were isolated from culture filtrates of A. alternata causing leaf spot on Dancy tangerine. The structure of toxin I was identified as 8-(N-2", 3", 4", 5",-tetrahydroxy-4", 6"-dimethyl-6"-octenoylvalyl)-9,10-epoxy-9-methyldecatrienoic acid. It had two geometric isomers: (2E,4Z,6E) type of decatrienoic acid for toxin Ib and (2E,4E,6E) for toxin Ic. Toxin II was the 5"-deoxy derivative of Ib. Ib at 10 ng/ml and IIb at 20 µg/ml induced veinal necrosis and a rapid increase in electrolyte loss from susceptible citrus leaves. Ib induced typical invagination of plasma membranes. Resistant citrus were not affected by toxins. Ib and IIb but Ic were released on spore germination. Infection hyphae were formed when avirulent spores were inoculated on leaves along with a small amount of Ib. ACT-toxin Ib plays a key role as host recognition factor in the early pathogenesis.

A887

PECTINASE PRODUCTION BY ASPERGILLUS FLAVUS AND A. NIDULANS DURING INFECTION OF WOUNDED COTTON BOLLS AND IN CULTURE. R. L. Brown, T. E. Cleveland, P. J. Cotty, and J. E. Mellon, USDA, ARS, Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA 70124.

Pectinase production in culture by high and low virulent Aspergillus flavus strains and an A. nidulans strain was compared by isoelectric focusing with production in wound-inoculated cotton bolls and on dead cottonseed. In both culture and plant tissue, the high virulent A. flavus strain

produced three pectinase activities (P1, P2c, and P3), whereas the low virulent strain produced two (P1 and P3). The A. nidulans strain produced a pectate lyase in liquid culture containing pectin, but did not produce the enzyme in either the developing cotton bolls or dead cottonseed. Activity P2c was not catabolite repressed and its expression in cotton bolls may be related to virulence of A. flavus.

A888

INCOMPATIBLE INTERACTIONS BETWEEN PHYTOPHTHORA MEGASPERMA F. SP. GLYCINEA AND SOYBEAN CULTIVARS WITHOUT KNOWN EFFECTIVE RPS GENES. R. E. Wagner and H. T. Wilkinson. Dept. of Plant Pathology, Univ. of Illinois, Urbana, IL 61801.

Soybean cultivars without known effective Rps genes formed compatible and incompatible interactions with race 3 of Phytophthora megasperma f. sp. glycinea (Pmg) on taproots of aeroponically grown plants. The frequency of incompatible interactions depended on the cultivar's level of rate-reducing resistance, pathogen aggressiveness, inoculum concentration and temperature. The magnitude of the interaction between the cultivar Corsoy and Pmg formed a continuum from brown necrotic flecks that formed at the site of infection (incompatible) to an expanding lesion that eventually extended from the root tip to the cotyledon (compatible). The similarity between lesions that formed following incompatible interactions on cultivars with and without effective Rps genes suggest that the same biochemical mechanism(s) could be responsible for single-gene and rate-reducing resistance.

A889

VARIATION IN LESION TYPE FOLLOWING INCOMPATIBLE INTERACTIONS BETWEEN PHYTOPHTHORA MEGASPERMA F. SP. GLYCINEA AND SOYBEAN CULTIVARS WITH EFFECTIVE RPS GENES. R.E. Wagner and H.T. Wilkinson, Department of Plant Pathology, University of Illinois, Urbana, Illinois 61801.

The interaction between soybean cultivars with different Rps genes and Phytophthora megasperma f. sp. glycinea (Pmg) was investigated on taproots of aeroponically grown plants. Incompatible interactions resulted in one of two lesion types, designated 1 and 2. Type 1 lesions were characterized by brown necrotic flecks that formed at the site of infection. Growth of the taproot continued unimpeded. Type 2 lesions were similar in appearance to those formed following compatible interactions, except lesion expansion was discontinuous resulting in a significantly shorter lesion. Growth of the taproot was terminated. Lesion type depended upon the cultivar, Rps gene, Pmg race, inoculum concentration, pathogen aggressiveness, and temperature. Type 2 lesions could indicate inefficient elicitation of the hypersensitive response.

A892

SHOOT-TIP MUCILAGE AND DISTRIBUTION OF FUSARIUM LATERITIUM ON SWEETPOTATO. C. A. Clark, J. A. Wilder-Ayers, and S. W. Matthews, Dept. Plant Pathology & Crop Physiology, Louisiana Agric. Expt. Station, LSU Agricultural Center and Dept. of Botany, College of Basic Sciences, Louisiana State University, Baton Rouge, LA 70803-1720.

Fusarium lateritium, causal agent of chlorotic leaf distortion (CLD), was isolated from NaOCl-treated parts of sweetpotato vines. It was isolated most frequently from leaf primordia, immature (folded) leaves, and axillary buds; less frequently from apical meristems, mature leaf tissue, floral parts and true seed; and infrequently from cross sections of vine nodes from CLD-affected plants. It was isolated less frequently from apical meristems and leaf primordia of plants recovered from CLD. Using light microscopy, fungal hyphae were observed on the surface of but not within sectioned symptomatic leaf or node tissue. Using scanning electron microscopy (SEM), hyphae were observed in regularly scattered clumps on the surface of unfolded leaves. On shoot tips, hyphae were present in a mucilage-like layer which covered the surface of the apical dome and leaf primodia. A similar layer was observed on healthy mericlones free of the fungus.

A893

Infection of flowering dogwood (*Cornus florida*) by the anthracnose fungus, *Discula* spp. Brown, D.A., M.T. Windham, E.T. Graham, & R.N. Trigiano, University of Tennessee, Knoxville, TN 37901.

Discula infection of the flowering dogwood was observed with scanning electron microscopy. Detached leaves were sprayed with a *Discula* spore suspension (in phosphate buffer, pH 6.8) and placed in the growth chamber at 18C. Germination was observed after 24 hrs. After 4 days, germ tubes and hyphae extended across the leaf. Neither direct penetration of the leaf surface or penetration via stomates was observed; however, germ tubes/hyphae appeared to elongate toward one of the two trichome types. These hyphae grew in the surface depression below these unusual leaf hairs and toward the trichome/leaf surface juncture. Hyphal penetration following this phenomenon and its role in infection is being investigated.

A896

REGENERATION OF INTERSPECIFIC FUSION HYBRIDS OF NICOTIANA TABACUM AND N. REPANDA. P. B. Nguyen, M. E. Daub, and A. E. Jenns, Dept. of Plant Pathology, North Carolina State University, Raleigh, N. C. 27695.

Nicotiana repanda has resistance to many important tobacco diseases, but will not cross with cultivated tobacco (N. tabacum) by conventionl means. In order to transfer resistance genes to N. tabacum, N. repanda was first hybridized with N. sylvestris which crosses with both species. This hybrid was then hybridized with N. tabacum cv. NC2326 by protoplast fusion. Parental lines were transformed for kanamycin or hygromycin resistance using Agrobacterium vectors. Mesophyll protoplasts were isolated from the antibiotic-resistant parent lines and fused using polyethylene glycol. Hybrid calli were selected by plating on media containing both antibiotics. Shoots were regenerated from hybrid calli, and shoot hybridity was verified by analysis of isozymes of glutamate oxaloacetate transaminase. Following rooting, hybrid plants will be screened for virus and nematode resistance present in the N. repanda parent.

A898

PITH DISCOLORATION IS CORRELATED WITH FUNGAL ERGOSTEROL CONTENT IN ANTHRACNOSE STALK ROT OF MAIZE. A. Muimba-Kankolongo, G. C. Bergstrom, *W. Köller, and E. B. Nelson, Depts. of Plant Pathology, Cornell Univ., Ithaca, 14853 & *Geneva, NY 14456.

A study was conducted to determine the relationship between anthracnose stalk rot (ASR) and tissue colonization by the causal fungus, *Colletotrichum graminicola*, in maize hybrids Cornell 281 (susceptible) and B 37 x LB 31 (resistant). Plants were inoculated with 1 ml of a 5×10^5 conidia/ml suspension into a wound in the internode above the brace roots and scored for ASR 21 days later. To determine ergosterol levels in tissues infected with *C. graminicola*, the pith tissues were homogenized in dichloromethane-MeOH (1:1), and the unsaponified lipid residues extracted with hexane. Ergosterol was quantified by reverse phase HPLC at 282 nm. Ergosterol level was positively correlated with the extent of pith discoloration and recovery of the fungus from the respective internodes. Moreover, significantly less ergosterol was detected in infected B 37 x LB 31 tissues than in infected Cornell 281. Estimates of fungal colonization corroborate visual rating schemes for ASR.

A899

EVALUATION OF CAPSICUM SPP. FOR RESISTANCE TO PHYTOPHTHORA CAPSICI. G. L. Hartman and T. C. Wang, Asian Vegetable Research and Development Center, P.O. Box 42, Shanhua, Tainan 74199, Taiwan, ROC.

Evaluations of inoculations using a range of zoospore concentrations and plant ages were compared by a disease index (0 = no disease to 4 = dead plant). The disease index for 'Blue Star' (highly susceptible) was not significantly different between inoculations using 10^2, 10^3, 10^4, or 10^5 zoospores/ml at 24 days after inoculation, whereas PI 201234 (highly resistant) had no disease at concentrations up to 10^5 zoospores/ml. Three- to 13-week-old inoculated plants of 'Blue Star' were equally susceptible with similar disease development recorded from 4 to 24 days after inoculation. Five- and 7-week-old inoculated plants of 'Szechwan' (moderately susceptible) had similar disease indices, but were significantly different from younger and older inoculated plants. PI 201234 had no disease regardless of age. Ten of 1,041 accessions had ≥ plant survival compared to PI 201234.

A900

VIRULENCE FORMS OF *Ascochyta rabiei* ON CHICKPEA IN THE PALOUSE. Hamidullah Jan and M. V. Wiese. Plant Pathology/PSES, Univ. of Idaho, Moscow 83843.

Ascochyta blight, caused by *A. rabiei*, severely damages chickpea in N. Idaho and E. Washington. To supplement chickpea breeding efforts, the virulence spectrum of local *A. rabiei* isolates was investigated. Thirty-nine isolates of *A. rabiei*, collected from chickpea seeds, plants and residue were compared on 15 differential chickpea lines in the greenhouse. The resultant spectra of blight reactions gave evidence for up to 19 different virulence forms. Eight isolates were closely related to *A. rabiei* Race 3 from ICARDA. Other isolate characteristics such as pycnidial diameter, spore size, colony color and growth rate on agar were not related to virulence. Local chickpea breeding efforts may need to incorporate broad spectrum blight resistance to be successful.

A901

CHARACTERIZATION OF SLOW RUSTING COMPONENTS IN MAIZE INBREDS AND SINGLE CROSSES. Z. Ngoko, R. A. Frederiksen, J. Craig, and J. D. Smith, Department of Plant Pathology and Microbiology, and Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843.

Puccinia polysora Underw., the incitant of southern corn rust, is the most devastating among the three rusts that occur on maize worldwide. Yield losses as high as 60% were reported. Presently, use of slow rusting varieties is the most effective means to control an epidemic. Altered infection type, reduced number of pustules, small area under the disease progress curve (AUDPC), shallow disease gradient, and reduced spread/unit of time were factors used to characterize slow rusting. Consistent results were obtained using the Gompertz transformation for each cultivar, while the logistic model gave variable results. Significant correlations were found between the infection type and yield loss, the pustule number and yield loss, and between the AUDPC and yield loss.

A903

THE ROLE OF GLUMES OF SORGHUM IN RESISTANCE TO GRAIN MOLD. S. B. Mansuetus, R. A. Frederiksen, R. D. Waniska, G. N. Odvody, and J. Craig, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

Isolates of *Fusarium moniliforme* from different sorghum plant parts were virulent in causing grain mold (GM) on RTx430. Vacuum

inoculation gave a higher GM and threshed grain mold rating and lower percent germination and kernel weight than did the spray inoculation. Colony forming units (CFU) on glumes of cultivars at boot stage of growth were greater on susceptible cultivars than on resistant cultivars. GM and CFU were positively correlated. Glumes at boot stage of growth of inoculated and noninoculated susceptible cultivars were colonized two days after inoculation whereas colonization was absent on glumes of a resistant cultivar. GM was negatively correlated with glume cover, glume length and glume area. Glumes of resistant cultivars had a physical and a chemical (free phenolic compounds) protective role in impeding penetration by *F. moniliforme*, thereby delaying the pathogens access to the developing caryopses. It appeared that less aggressive isolates are selected against while more aggressive isolates reach the caryopses easily, particularly with susceptible cultivars.

A904

DETECTION OF SNOW MOLD RESISTANT PROPERTIES IN WINTER WHEAT PLANTS AND ANDROGENIC PLANTLETS. J. H. McBeath and F. Mehdizadegan. Agricultural and Forestry Experiment Station, University of Alaska Fairbanks, Fairbanks AK 99775-0080.

A method was developed to assess the effects of extracellular enzymes of Sclerotinia borealis and sclerotial low temperature basidiomycete on winter wheat. Leaf segments, taken from cultivars Capitan, Froid, Roughrider and Blizzard and from plantlets derived from the anther cultures were weighed and treated with various concentrations of snow mold extracellular enzymes (consisting mainly of cellulolytic and pecteolytic enzymes). After a 7-day incubation at 10 C, the chlorophyll content in the leaf segments was extracted and measured. Gradients of responses were observed of cultivars and plantlets ranging from no change to chlorosis. Enhancements in resistance to snow mold enzymes were observed in plantlets derived from Roughrider, which possess moderate snow mold resistance properties.

A905

RESISTANCE OF MAIZE KERNELS TO DECAY BY ASPERGILLUS FLAVUS. J. M. Rivera and C. A. Martinson, Department of Plant Pathology, Iowa State University, Ames, IA 50011.

Maize kernels were inoculated with A. flavus spores and stored in a 92.5% RH (over 2.25 molal NaOH) and 33.0 C environment. Germplasm was ten inbred lines selfed in five environments and all possible crosses of the lines. Kernel germination and seedling emergence from a sand-soil medium was the measure of resistance. Resistance ranking of the inbred lines was B84(best), B77, Mo40, B76, B79, Mo17, B73, B85, Va35, and B14A. Environment of kernel production altered rankings slightly, but environment affected primarily the magnitude of the disease response. Inbred line response to A. flavus was transmitted to the hybrid and it predicted hybrid response best when used as the female parent. Resistance was strongly related to a maternal effect with B84 and B77 the better female parent in hybrids. Resistance of maize kernels to A. flavus attack in a high humidity, high temperature storage environment is a genetically controlled trait that is influenced greatly by environment of kernel production.

A906

IMPROVEMENT IN RESISTANCE TO ASPERGILLUS FLAVUS IN AN ANTIGUA POPULATION OF MAIZE. C. A. Martinson*, J. M. Rivera*, and A. R. Hallauer°, Department of Plant Pathology* and Department of Agronomy°, Iowa State University, Ames, IA 50011.

Maize kernels were inoculated with A. flavus spores and stored in a 92% RH environment (over either 2.25 molal NaOH or saturated KNO_3). Kernel germination and seedling emergence was the measure of resistance. A CIMMYT population from Antigua was mass selected for earliness for 4 years, and then kernels from 92 selfed ears were assayed for resistance. Emergence ranged from 0-88%, genetic coefficient of variability was 115.4%, and predicted heritability was 96.6%. Recurrent S_1 selection (10% selection intensity) was performed for two cycles and all cycles were evaluated. Emergence was 40.3, 59.8, and 80.0% for cycles 0, 1, and 2, respectively. Seed of the base population was inoculated, stored at 92% RH, and then planted in the field. Surviving plants were randomly intermated and equal numbers of kernels from each ear were mixed and recycled. Germination after A. flavus exposure in storage increased from 31.5% in cycle 0 to 47.1% in cycle 2. Heritability of resistance was established.

A907

INCORPORATION OF SUGARCANE MOSAIC VIRUS RESISTANCE INTO SUGARCANE FROM FERAL GERMPLASM. M. P. Grisham and B. L. Legendre, USDA, Agricultural Research Service, Sugarcane Research Unit, P. O. Box 470, Houma, Louisiana 70360.

A basic sugarcane breeding program was initiated at Houma, LA in 1964 with the primary objective to develop cultivars resistant to sugarcane mosaic virus (SCMV). Crosses were made with diverse germplasm of feral or wild Saccharum species, primarily S. spontaneum, and related genera. Since 1986, clones (F_1 or BC_{1-4} progeny) selected from this program and assigned permanent breeding germplasm (US prefix) or candidate cultivar (CP prefix) designations have been examined for natural infection by SCMV in the field. In the pedigrees of the 83 US and 15 CP clones examined, only 2% of the recurrent parents were resistant to SCMV; however, 56% of the progeny were rated resistant and 44% were rated susceptible based on visual symptoms. The data suggest that feral germplasm may be used in sugarcane breeding to increase the frequency of resistance to SCMV.

A909

INVESTIGATION OF BACTERIAL FRUIT BLOTCH OF WATERMELON IN INDIANA. K. K. Rane and R. X. Latin, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN, 47907.

Bacterial fruit blotch severely affected watermelon crops on several Indiana farms in 1989. The pathogen, tentatively identified as Pseudomonas pseudoalcaligenes subsp. citrulli (Ppc), caused rapidly expanding watersoaked lesions on the surface of maturing watermelon fruit. The pathogen also caused small necrotic lesions on foliage. Seed obtained from symptomatic fruit were planted in the greenhouse and more than 40 % of the resultant seedlings developed watersoaked lesions on cotyledons. Bacterial strains recovered from cotyledons were similar to those collected from infected fruit. Rind from infected fruit was buried outdoors in soil and sampled periodically to determine the ability of the pathogen to overwinter in midwestern fields. Ppc was recovered 1 week after burial, but attempts to recover the pathogen after 3 and 4 months were not successful.

A910

A NEW RACE OF THE TOMATO GROUP OF STRAINS OF XANTHOMONAS CAMPESTRIS pv. VESICATORIA. J. F. Wang, J. B. Jones, J. W. Scott, and R. E. Stall. Plant Pathology Department, Univ. of Florida. Gainesville, Fl 32611

A strain of X. c. pv. vesicatoria from Brazil (Xv 56) caused disease in the tomato breeding line Hawaii 7998 (H7998), a line resistant to bacterial spot in Florida. It was not virulent on pepper and belongs to the tomato group of strains (XcvT). Electrolyte leakage patterns from susceptible leaves (Bonny Best) inoculated with Xv 56 and a typical strain of XcvT from Florida (Xv 75-3) were similar, but leakage occurred more rapidly after inoculation of leaves of H7998 with Xv 75-3 than with Xv 56. With initial inoculum of 10^5 cfu/ml the growth curves of Xv 56 in H7998 and Bonny Best were similar and populations reached almost 10^9 cfu/cm^2 in 6 days. The growth curve of Xv 75-3 reached its maxi mum (10^6 cfu/cm^2) in 2 days in leaves of H7998 and in 3 days (10^7 cfu/cm^2) in leaves of Bonny Best.

A911

OVERWINTERING OF CLAVIBACTER MICHIGANENSIS SUBSP. MICHIGANENSIS AND SPREAD ON ALTERNATIVE HOSTS AND NON-HOST PLANTS. R. J. Chang, S. M. Ries, and J. K. Pataky. Department of Plant Pathology, University of Illinois, Urbana, Illinois 61801.

Overwintering of Clavibacter michiganensis subsp. michiganensis (CMM) associated with tomato (Lycopersicon esculentum) stems, and spread of CMM on alternative hosts and non-host plants were evaluated with rifampin-resistant mutants and a selective medium. The bacterium was recovered after 196 days from tomato stems placed on the soil surface and from stems buried 10, 20, and 30 cm. The survival rate was highest on the soil surface, but there were no differences in survival at the 10, 20, and 30 cm depths. Viable cells decreased about 100 to 10,000-fold from November 1988 to May 1989. No pattern of secondary spread of CMM was detected on alternative hosts or non-host plants that were grown in tomato field. Symptoms of secondary infection were not observed on plants of nine other species, however epiphytic populations of CMM were higher on solanaceous plants than non-solanaceous species.

A912

RESPONSE TO ERWINIA INOCULATION IN TISSUE CULTURE PLANTLETS OF THREE POTATO CULTIVARS. C. Skroch, D. J. Gallenberg, and P. L. Spinski, Department of Plant Science, Box 2109, South Dakota State University, Brookings, SD 57007.

Plantlets of three potato cultivars differing in their field reaction to blackleg caused by Erwinia carotovora spp. atroseptica (Eca) were grown on both standard MS and high calcium media for 4-5 weeks prior to stem inoculation with one of two Eca strains. After one week, disease severity was rated using a 0-3 scale. Approximately 55 plantlets were inoculated in each of the 12 treatments (cultivar x medium x Eca strain), and data were analyzed using SAS PROC CATMOD. Among cultivars, the field susceptible 'Norchip' had the lowest overall disease severity, followed by 'Red Pontiac' (moderately resistant), with 'Russet Burbank' (resistant) showing the greatest severity. While a number of factors may contribute to this departure from field observations, it may be explained in part by tissue analyses which indicated significantly higher levels of calcium in 'Norchip' compared to 'Russet Burbank'.

A913

INFLUENCE OF GROWTH MEDIUM ON RESPONSE OF POTATO TISSUE CULTURE PLANTLETS TO ERWINIA. C. Skroch, D. J. Gallenberg, and P. L. Spinski, Department of Plant Science, Box 2109, South Dakota State University, Brookings, SD 57007.

Potato tissue culture plantlets were grown on both standard MS and high-calcium media to determine the effects of medium nutrient levels on response to Erwinia. After 4-5 weeks growth on the two media, plantlets were stem-inoculated in vitro with Erwinia carotovora spp. atroseptica (Eca) and observed after one week for disease severity. Across the three cultivars and two Eca strains used, disease severity was significantly lower in plantlets grown on the high-calcium medium as compared to standard medium plantlets. Tissue analyses indicated significantly greater levels of calcium in stem and leaf tissue of plantlets grown on the high-calcium medium. Increased calcium levels likely play a role in the lower disease severity. However, tissue analyses also indicated significantly greater levels of chloride in the same plantlets. Chloride has been shown to reduce disease severity in some fungal disease systems, but its interaction with bacterial diseases is not known.

A914

USE OF POLYMERASE CHAIN REACTION TO DETECT PATHOGENIC ISOLATES OF AGROBACTERIUM. L-C. Dong[1], K. L. Thies[2], D. S. Luthe[1], and C. H. Graves, Jr.[2]. Department of Biochemistry and Molecular Biology[1] and Department of Plant Pathology and Weed Science[2], Mississippi State University, Miss. State, MS 39762.

The polymerase chain reaction (PCR), a very sensitive tool for the identification of specific regions of DNA present in small quantities, was used to detect the presence of T-DNA in 39 Agrobacterium isolates from Vitis spp. Oligonucleotide primers were homologous to the T-DNA regions from Agrobacterium tumefaciens Ag63 and amplified a 150 bp region. Twenty-three of the 39 isolates tested contained T-DNA based on the PCR results. In most cases the PCR results confirmed pathogenicity tests using detached leaves from Agrobacterium-free muscadine plants. Our results indicate that the PCR is a specific, sensitive and suitable technique for distinguishing potentially pathogenic isolates of Agrobacterium.

A915

USE OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS TO CHARACTERIZE STRAINS OF *PSEUDOMONAS SOLANACEARUM*. Elizabeth Barlow, Douglas Cook, and Luis Sequeira. Department of Plant Pathology, University of Wisconsin-Madison, Madison WI 53706.

A total of 150 strains of *Pseudomonas solanacearum* from a world wide range of hosts and geographical locations and representing all known biovars and races were examined by restriction fragment length polymorphism (RFLP) analysis. Double digests (EcoR1/BamH1) of total genomic DNAs were electrophoresed, blotted onto nylon membranes and hybridized with nine DNA probes. Seven of these probes encode regions required for virulence and induction of the hypersensitive reaction. In general, the RFLP patterns were as previously reported for each of the corresponding biovars, although there were many evident polymorphisms among strains. Strains were grouped according to similarity coefficients calculated on the basis of number of DNA fragments in common. The usefulness of the method was demonstrated by our ability to characterize several unknown strains from heliconias imported into Australia as belonging to the SFR group of race 2. This group is highly pathogenic to bananas and probably originated in Venezuela.

A916

SANITATION OF CAPRIFIGS (MALE FIGS) REDUCES FIG ENDOSEPSIS CAUSED BY FUSARIUM MONILIFORME VAR. FICI IN CALIMYRNA FIGS. Themis J. Michailides and D. P. Morgan, Dept. of Plant Pathology, Univ. of Calif., Berkeley/Kearney Ag. Center, 9240 S. Riverbend Ave., Parlier, CA 93648.

Fungicide dip or spray treatments of the mamme crop (winter crop) reduced the incidence of Fusarium spp. on the mammes and on the emerged fig wasps (Blastophaga psenes), but not of other organisms. The effects of mamme treatments on the disinfestation of profichis (spring crop) and on the emerged wasps varied according to the fungicide treatment. In all experiments untreated selected symptomless mammes showed the lowest incidence of F. moniliforme on fruit tissues and resulted in the lowest percentage of contaminated profichis, emerged wasps, and Calimyrna figs. Fungicide treatments of mamme figs resulted in cleaning of emerged fig wasps. Recontamination of pathogen-free wasps by contaminated plant surfaces of caprifig and smyrna fig trees could explain the high variability and the lack of effectiveness of fungicide treatments in controlling fig endosepsis in profichi and Calimyrna crops.

A917

CERATOCYSTIS LIMB CANKER OF ALMOND. B.L. Teviotdale and D.H. Harper. University of California, Kearney Agricultural Center, Parlier, California 93648.

Ceratocystis fimbriata was identified as the causal agent of a limb canker of almond trees. Natural infections occur at twigs or small pruning cuts, and multiple cankers girdle and kill branches. Pruning cuts 0, 2, 4, 7, and 14 days old inoculated with a spore suspension of C. fimbriata in November, December, January, and February developed cankers at all sites except 7 and 14 day-old cuts inoculated in November. Cankers were largest at sites inoculated immediately after cuts were made. Infections occurred at small wounds, made by breaking twigs or puncturing the bark with a lancet, when inoculated in alternate months throughout the year. Inoculation of superficial wounds or those that penetrated the bark midway or fully to the cambium resulted in 0, 66, and 84% infection, respectively.

A918

ISOLATION AND PATHOGENICITY OF ALTERNARIA LIMICOLA ASSOCIATED WITH CITRUS LEAF SPOT IN MEXICO. **M.E. Palm, USDA/APHIS and E.L. Civerolo, USDA/ARS, BARC-West, Beltsville, MD 20705-2350.**

Alternaria limicola, a newly described fungus associated with a leaf spot disease of Citrus in Mexico, was isolated from 15 of 16 samples of six Citrus spp. collected in 1989 and 1990 in Colima. Two bacterial strains resembling Xanthomonas campestris were isolated from only one sample. Mexican lime and 'Duncan' grapefruit seedlings were artificially inoculated by spraying the terminal foliage with aqueous suspensions containing either 200-400 conidia/ml of A. limicola or 108 cfu/ml of X. campestris. Lesions similar to those observed in the field developed and the fungus was reisolated from all inoculated plants. Neither of the xanthomonad strains produced any symptoms. This confirms previous work done in Mexico that the primary cause of citrus leaf spot is A. limicola and not a bacterium.

A919

COMPARISONS OF HICKORIES INFECTED WITH CLADOSPORIUM CARYIGENUM USING SCANNING ELECTRON MICROSCOPY. S. V. Diehl and C. H. Graves, Dept. of Plant Pathology and Weed Science, Mississippi State University, MS State, MS 39762.

Leaves of Schley, Stuart, Success and Stevens pecan cultivars and Mockernut hickory plus nut husks from Schley and Stuart were compared for differences in surface morphology and fungal development in scab-infected and non-infected tissues with scanning electron microscopy. Internal tissue structure was also compared by freeze fracturing some samples. Scab lesions on both nuts and leaves of the susceptible Schley cultivar consisted of dense, compact mats of hyphae with prolific sporulation and complete breakdown of spongy and palisade parenchyma structure. Leaf lesions on the resistant Mockernut hickory had sparse mycelia, no observed sporulation and minimal disruption of both surface and internal tissue structure. The reaction of other cultivars varied between these two extremes. No fungal hyphae were seen within intact or degrading tissues of leaves or nut husks. Hyphae were observed only on tissue surfaces or sub-cuticularly.

A921

ASSOCIATION OF NECTRIA RUGULOSA WITH QUICK DECLINE OF MACADAMIA TREES. W. H. Ko and R. K. Kunimoto, Department of Plant Pathology, University of Hawaii, Hilo, Hawaii 96720.

In 1986, a number of macadamia trees at Keaau on the island of Hawaii died within few months after the appearance of decline symptoms. Initial symptoms were yellowing and browning of some leaves within the tree canopy. The number of affected leaves increased rapidly and within few months leaves on the entire tree turned brown and the tree died. When 23 declining trees at Keaau were closely inspected, 12 had with numerous small reddish perithecia on the trunks. An ascospore culture was identified by C.A.B. International Mycological Institute as Nectria cf. rugulosa with Cylindrocarpon anamorph. When the fungus grown in a wheat-oat medium was used to inoculate branches of healthy macadamia trees, 10% of inoculated branches were infected after 3 months. The number of infected branches increased to 50% 10 months after inoculation. All of the control branches remained healthy. N. rugulosa was reisolated from all of the diseased branches.

A922

ASSOCIATION OF XYLARIA ARBUSCULA WITH QUICK DECLINE OF MACADAMIA TREES. W. W. Ko and R. K. Kunimoto. Department of Plant Pathology, University of Hawaii, Hilo, Hawaii 96720.

Fruiting bodies of several species of fungi were frequently observed on the trunks of dying or dead macadamia trees in the areas affected by quick decline at Keaau on the island of Hawaii. Club-shaped carbonaceous fruiting bodies identified by J. D. Rogers as Xylaria arbuscula were found on about 50% of the declining macadamia trees. Wood tissues beneath the fruiting bodies showed extensive decay and contained black zone lines. The colony appearance of a fungus frequently isolated from diseased tissues was identical to that of X. arbuscula culture derived from an ascospore. When the fungus grown on a wheat-oat medium was used to inoculate branches of healthy macadamia trees, 40% of inoculated branches with part of the bark removed were infected after 4 months, while 80% of inoculated branches with bark gently scraped were infected during the same incubation period. All the control branches remained healthy. X. arbuscula was reisolated from all the diseased branches.

A923

ELIMINATION OF BANANA BUNCHY TOP VIRUS FROM DISEASED BANANA TISSUES. R. Y. Wu and H. J. Su, Development Center for Biotechnology, 81 Chang Hsing St., Taipei, Taiwan, and Department of Plant Pathology and Entomology, National Taiwan University, Taipei, Taiwan.

When banana bunchy top virus (BBTV)-infected tissues were cultured at 35 C, some of the buds started to produce healthy roots and developed into healthy-appearing plantlets after 3 months and 5 of 11 tissues tested produced healthy-appearing plantlets in 6 months. The crude extract from healthy-appearing plantlets did not show any activity when assayed with monoclonal antibody against BBTV. When heat-induced healthy plantlets were inoculated with viruliferous aphids, all of them developed bunchy top symptoms indicating that these plantlets were not resistant to BBTV. Results suggested that uneven distribution of virus at low concentration at high temperature may give rise to BBTV-free primordial cells which in turn may develop into healthy plantlets.

A924

COLLOIDAL GOLD LOCALIZATION OF SPIROPLASMA CITRI SURFACE PROTEINS. J. Fletcher and C. Colambage, Dept. of Plant Pathology, Oklahoma State University, Stillwater, OK 74078.

Polyclonal antibodies specific for four surface proteins (29, 58, 77, and 89 KDa) of Spiroplasma citri were used to characterize the prevalence and distribution of these proteins on the membrane. Electron microscopy and colloidal gold labeling techniques were applied to whole and sectioned cells for ultrastructural analysis of the proteins. The random distribution of gold particles all along the surface of the cells indicated that p29 (spiralin), p77, and p89 are not clustered or arranged in a recognizable pattern. The results for the p29 agree with previous reports; p77 and p89 have not been previously studied. The p29 was more prevalent than p77 or p89. The prevalence and distribution of gold particles in p58 samples did not differ from controls treated with preimmune serum; therefore, the p58 antibodies, which were made to protein isolated from SDS gels, may not recognize the native protein.

A926

THE BEET LEAFHOPPER TRANSMITTED VIRESCENCE AGENT CAUSES A PREMATURE FLOWERING AND VIRESCENCE DISEASE OF CARROTS. M. E. Shaw, B. C. Kirkpatrick, R. M. Davis and *D. A. Golino. Department of Plant Pathology, University of California, and *USDA/ARS, Davis, CA 95616.

During 1988/89 a premature flowering disease of carrots was observed in several fields located in the Southern San Joaquin Valley. Roots of diseased plants were woody and unmarketable and most infected plants produced virescent flowers. Symptomatic plants tested positively by both DNA hybridization assays using cloned fragments of BLTVA extrachromosomal DNA as probes and a BLTVA-specific, enzyme-linked immunosorbent assay. BLTVA-MLOs were transmitted from diseased carrots to herbaceous indicator plants using Circulifer tennelus leafhoppers. Southern blot analyses of undigested DNA from diseased carrots showed there was tremendous diversity in the numbers and sizes of plasmids in the field-collected, BLTVA-infected carrots.

A927

A SURVEY OF PLANT PATHOGENIC MOLLICUTES FOR THE ABILITY TO CAUSE THE HOST INDUCTION RESPONSE. D. A. Golino*, V. Butler*, and M. Shaw. USDA-ARS*, Department of Plant Pathology, University of California, Davis, CA 95616.

The beet leafhopper transmitted virescence agent (BLTVA) line FC-83-13 has been demonstrated to cause flowering in plants grown under environmentally non-inductive conditions, an effect known as the host induction response (HIR). Three new lines of BLTVA, three lines of aster yellows, a line of western-x and Spiroplasma citri strain -215 were screened for their ability to cause the HIR. Each pathogen was used to inoculate groups of biennial radish, celery or chinese cabbage, all of which have been shown to exhibit the HIR. All of the BLTVA lines and none of the other mollicutes were demonstrated to cause the HIR.

A929

OCCURRENCE OF TOMATO SPOTTED WILT VIRUS IN COLORADO. R. D. Koski, W. M. Brown, Jr., and H. F. Schwartz. Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, Colorado 80523

In 1988, Tomato Spotted Wilt Virus caused diseases of begonia, eggplant, and tomato. The virus was not known to exist in Colorado prior to 1988. During 1989, the Plant Disease Clinic received 14 plant samples infected with Tomato Spotted Wilt Virus. The 1989 Colorado host list for Tomato Spotted Wilt Virus included coreopsis, eggplant, eustoma, gerbera, pepper, and tomato. The Agdia, Inc. PathoScreen^{TM} ELISA test kit specific for Tomato Spotted Wilt Virus was used to confirm the presence of the virus in plant tissues. Agdia, Inc. now has PathoScreen^{TM} kits available for detection of the Impatiens and Lettuce Isolates of Tomato Spotted Wilt Virus. These kits will be used during 1990 to screen suspected Tomato Spotted Wilt Virus-infected plants to determine if both strains are present in Colorado.

A930

Isolation of *Discula* spp. from anthracnose-infected Chinese dogwoods. Brown, D.A., M.T. Windham, and R.N. Trigiano, University of Tennessee, Knoxville, TN 37901.

Chinese dogwoods have been considered resistant to dogwood anthracnose and a source of disease resistance. However, leaves, stems, and twigs which exhibited anthracnose symptoms were obtained from Chinese dogwoods in 1989. Characteristic *Discula* -like acervuli

developed within 72 hrs on this diseased tissue. These acervuli were shown to contain *Discula* -like conidia and subcultures were taken. These isolates are similar to those from flowering dogwoods in morphology, growth rate, and sporulation. Pathogenicity of these isolates to flowering dogwoods is under investigation. These results suggest that while Chinese dogwoods may prove more anthracnose-resistant than *Cornus florida* cultivars, *C. kousa* should be screened before being incorporated in the breeding program.

A932

A POLYMERASE CHAIN REACTION FOR DETECTION OF THE TAKE-ALL FUNGUS, GAEUMANNOMYCES GRAMINIS, IN INFECTED WHEAT PLANTS. Kurt Schesser and Joan M. Henson. Dept. of Microbiology, Montana State University, Bozeman 59717

The sequence of a DNA fragment that was previously found to be specific for Gaeumannomyces was determined. Polymerase chain reaction (PCR) was used to amplify this sequence in wheat seedlings infected with Gaeumannomyces graminis var. tritici which allows detection of fungal DNA in infected tissue without culturing the fungus. Other applications using PCR for Gaeumannomyces graminis detection will be discussed.

A933

The Characterization of an Infectious cDNA Clone of Potato Virus X

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To analyze the mechanism of coat protein mediated protection and the molecular events during PVX infection, a full-length cDNA encoding the entire genome of potato virus X (PVX) has been isolated. This cDNA was placed behind the bacteriophage T7 promoter, which allowed infectious PVX transcripts to be produced *in vitro*. These transcripts were inoculated onto a local lesion host, *Chenopodium amaranticolor*, and a systemic host, *Nicotiana tabacum*. Lesions appeared on these plants that were typical of PVX infection, although infectivity was lower when compared to authentic PVX RNA. Electron microscopy of lesions isolated from infected plants showed typical flexuous rods of PVX. We are currently improving the infectivity of the clone by removing extraneous DNA from the transcription vector. We are using infectious transcripts to study interactions between coat protein in transgenic plants and viral RNA. In addition, we are studying the functions of various open reading frames encoded by the PVX genome.

A934

EVALUATION OF SOYBEAN LINES REGENERATED FROM ORGANOGENIC CALLUS FOR THEIR REACTION TO SEPTORIA GLYCINES. H. S. Song, S. M. Lim, and J. M. Widholm. Departments of Plant Pathology and of Agronomy and USDA-ARS, University of Illinois, IL 61801.

Soybean plants resistant to the pathotoxic culture filtrates of Septoria glycines were regenerated in vitro. In 1989, more than 700 R_2 (second selfed generation) were evaluated for their reaction to S. glycines by inoculating the plants in the field. Fifteen percent of the inoculated plants did not produce brown spot symptoms until the R6 reproductive growth stage. At harvest, the severity of brown spot on these plants was less than 10%. Brown spot developed in the other inoculated plants earlier and the severity ranged from 75 to 100% at the R6 growth stage. R_3 lines from all of the R_2 plants will be evaluated in the field.

A935

Purification and characterization of chitinases and β-1,3-glucanases from *Beta vulgaris* leaves infected with *Cercospora beticola*

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Many plants respond to infection with a pathogen by producing a number of proteins. Among these defence related proteins are the hydrolytic enzymes, eg. chitinases and β-1,3-glucanases.
We have studied the interaction between *Beta vulgaris L.* and the leaf pathogen *Cercospora beticola*.
It is shown that both chitin and β-1,3-glucan are constituents of the cell wall of *Cercospora*. Radioactive labelled N-acetylglucosamine and glucose are specificially incorporated into the cell wall of growing hyphae and may be removed from the apex by treating the hyphae with hydrolytic enzymes.
Chitinase and β-1,3-glucanase activities are strongly induced following infection with *Cercospora*. From infected leaves nine chitinases and five β-1,3-glucanases have been purified to homogeneity by affinity column chromatography followed by cation exchange chromatography on a FPLC system. Amino acid compositional analysis, N-terminal and partial amino acid sequencing have been carried out on the major enzymes.
Antibodies have been raised against three chitinases and two β-1,3-glucanases. Two serologically different groups of chitinases are present in sugar beet.